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A Comparison of the β-Lectins from Douglas-Fir and Loblolly Pine During Growth from Seed to Sapling

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A COMPARISON OF THE β-LECTINS FROM DOUGLAS-FIR AND LOBLOLLY PINE DURING GROWTH FROM SEED TO SAPLING

A thesis submitted by

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ABSTRACT

The presence of β-lectins, as defined by their precipitation with Yariv glucoside [1,3,5-tri-(p-β-D-glucosyloxyphenylazo)-2,4,6-trihydroxybenzene] was confirmed in the seeds of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] and established in the seeds of loblolly pine (Pinus taeda). The characterization of multiple samples of β-lectin isolated from six developmental states in each species revealed structural variations relatable by a two-way analysis of variance to the state and species of the sample's origin. Sampling was from the dry seed, stratified seed, cotyledon seedling, two-month-old seedling, sapling, and callus states.

The β-lectins of both species showed similar developmental trends. The sedimentation coefficient and weight percent protein increased in β-lectins from the dry seed to cotyledon seedling states and decreased thereafter with the maxima of the sedimentation coefficients and near minima of the percent protein occurring in the calli β-lectins. The carbohydrate moieties of the β-lectins showed a gradual decrease in galactose relative to the minor sugars with successive developmental states. The neutral sugar composition of the calli β-lectins most closely resembled that of the β-lectins from the cotyledon seedlings.

For any given developmental state, the mean β-lectin sedimentation coefficient and weight percent protein of loblolly pine was less than that for Douglas-fir. Loblolly pine β-lectins tended to be richer in neutral sugars (especially galactose) than those from Douglas-fir. Loblolly pine β-lectins also tended to have a lower hydroxyproline and higher aspartate content than those from Douglas-fir. Infrared spectra seem to corroborate some of these interspecies differences.

The amino acid compositions of the β-lectins exhibited great stability over all samples analyzed. Nineteen residues were present at approximately the same relative concentrations as those of the gymnosperm species reported in the literature.

Similarly, the electrofocusing patterns of the β-lectins changed little from sample to sample.

No mutually significant differences could be found in β-lectins isolated from the needles, stems, and roots of saplings from the two species.

These results demonstrate not only that the β-lectins satisfy the informational molecule requirements of presence and variability, but also establish a precedent for their use as developmental and taxonomic markers.

INTRODUCTION

A WORKING DEFINITION OF THE β-LECTINS

The β-lectins are a class of arabinogalactan-proteins widely distributed throughout the plant kingdom (1,2). They have been found in tissue from the various parts of plants as well as from a large number of distantly related species from liverwort to sycamore. This near ubiquity of the β-lectins implies that they may serve some vital function in the lives of plants. Unfortunately, no such function has yet been demonstrated.

These arabinogalactan-proteins are called β-lectins by analogy. Lectins are proteinaceous natural products that noncovalently bond to specific carbohydrate residues (3,4), as in the bonding of D-galactose to ricin (the lectin isolated from castor beans, Ricinus communis). The β-lectins are arabinogalactan-proteins synthesized by plants that noncovalently bond to the Yariv β-D-glycopyranosides (1), hence their name, meant to convey that they are "β-D-glycopyranosyl Yariv compound bonding lectins." The structure of the Yariv β-D-glucopyranoside is given in Fig. 3, but all Yariv compounds have the same triskelion-shaped core aglycone and differ from one another only in the nature of their glycosyl substituents, R (cf. Fig. 4). The name "β-lectin" is used throughout this thesis exclusively for those compounds which have been observed to bond to the Yariv β-D-glycopyranosides, since not all arabinogalactan-proteins (AGPs) exhibit this behavior (5). In addition to the foregoing definition, nomenclature and notation for the glycosylphenylazo dyes throughout this thesis follows that of Jermyn (1), as explained in the Glossary.

THE DISCOVERY OF THE β-LECTINS

The first suggestion in the literature of the existence of the β-lectins came in 1967 when the Yariv glucoside (β-GLU) was used to precipitate a polysaccharide present in the seed extract of Lotus tetragonolobus that was interfering with the

isolation of an L-fucose binding protein (6). Five months later a follow-up report appeared that contained a preliminary characterization of the β-GLU precipitates from arabic acid and three seed polysaccharides (7). The carbohydrate contents for the seed polysaccharides were 70% (jack bean meal), 80% (soya bean flour), and 82% (maize flour). Paper chromatography of these polysaccharides' acid hydrolyzates gave two major spots corresponding to galactose and arabinose.

Sometime later, Jermyn noted the presence of two precipitin lines in a double-diffusion test of Yariv glucoside (β-GLU) against a seed extract from jack bean (Canavalia ensiformis). One line was due to the precipitation of β-GLU by the lectin concanavalin A. Further double-diffusion tests proved the second line to be identical to that formed by Yariv glucoside reacting with some component in a large sampling of different seed extracts. A short notice of this discovery (8) was given some seven years after the first suggestion of the lectin's existence (6). A few months later, early in 1975, a major paper (1) was published which defined the β-GLU precipitates as a class of compounds possessing the same bonding specificity and similar physicochemical properties and existing in a large variety of seed plants. In agreement with the first report (7) on these β-GLU precipitates, it was found that they had a high carbohydrate content (80-90%), with the major sugars being galactose and arabinose. Going beyond that earlier report was the discovery that the β-GLU precipitates contained a protein moiety of remarkably constant composition, thus establishing a biochemical link between them and the lectins (specific sugar-bonding proteins).

Since the publication of Jermyn's seminal paper, active research has continued on the β-lectins throughout the world. The bulk of this research has been done in Australia and New Zealand, although reports have also come from England, Japan, the Netherlands, and the United States. The following section briefly reviews the results obtained by these research efforts in order to put the contribution of this thesis into context.

LITERATURE REVIEW

OCCURRENCE OF THE β-LECTINS

IN THE PLANT KINGDOM

The paper which defined the β-lectins reported their presence in the seeds of 91 out of 104 families tested (1). The families were sampled from the gymnosperms and angiosperms (both monocots and dicots). Subsequent research has shown that the handful of negative results does not necessarily imply the absence of the β-lectins from the species tested (9). On the contrary, it now seems likely that the β-lectins are present in all spermatophytes (seed-bearing plants) although their concentration in a given tissue varies greatly depending on the source of the plant material (species, plant part, and developmental state sampled).

These distribution studies were continued in a later publication (2) which, in addition to sampling new spermatophytic species, also extended the survey backward in time in an evolutionary sense. Representatives of both the pteridophytes (ferns and fern-allies) and bryophytes (mosses and liverworts) were discovered to contain β-lectins by double-diffusion precipitin tests. Confirmation that the precipitin products of these lower plants were related to the β-lectins of the spermatophytes came through comparison of two amino acid analyses (one from a fern species, Cyathea australis, and one from a moss species, Polytrichum juniperinum) with the β-lectin amino acid analyses of a dozen seed-bearing species.

All of the major divisions of the subkingdom Embryophyta have thus been shown to contain β-lectins. It seems that the β-lectins may have existed for more than 500 million years (since the early Paleozoic Era), their advent perhaps coinciding with the emergence of the first land plants (terrestrial autotrophic embryophytes). Given

this wide distribution in the plant kingdom and evolutionary stability, it is not surprising that some basic physiological role is being sought for the β-lectins.

IN PLANT PARTS

Although the distribution study which established the ubiquity of the β-lectins in the spermatophytes was conducted via precipitin tests on seed extracts, homogenates of other plant tissues were also examined (1). The β-lectins were found in all the major parts of a plant (both the aboveground and underground plant parts). In general, the precipitin reaction in a species' tissues was weaker than that for its seeds. Using crude plant homogenates (tissue ground in phosphate-buffered saline solution without any pretreatment), β-lectins were not noted in the underground parts of two of the three angiosperms tested. In the second distribution study (2), it was discovered that many plant tissues would not give a positive precipitin test without a preliminary hot ethanolic extraction. However, following such extractions, β-lectins actually were isolated from nonseed tissues of higher plants (leaves, pods, and petioles) and lower plants (fern leaves and moss total green tissue). These studies indicate that, in addition to being present in most plants, the β-lectins may be continuously present through all parts of a plant during its lifetime.

IN PLANT CELLS

The first application of the Yariv glucoside as a cytological stain (10) came almost immediately following the preliminary announcement of the β-lectins' existence (8). Specific staining was observed for β-GLU or β-GAL which was absent in control sections pretreated with salicin (o-hydroxymethylphenyl-β-D-glucopyranoside) and in control sections stained with α-GAL or a α-MAN (see Glossary for an explanation of Yariv compound notation). Three species of legume seed exhibited staining in the intercellular spaces (vesicle-associated), cell walls, and peripheral cytoplasm of cotyledon parenchyma cells. However, another study (11) using the same

staining methods, did not find this primarily extracellular distribution of the β-lectins evident in the seeds of three cereal species (ryegrass, barley, and wheat). Instead, the aleurone layer cells of these grains stained, especially at the cytoplasm/cell wall interface; for the ryegrass, staining also occurred in the endosperm tissue (between the subgranules of multilocular starch granules). Faint staining was also noted in the walls of suspension-cultured ryegrass endosperm cells and in sections of endosperm tissue that had been prewashed in ethanol.

Staining studies employing the Yariv glucoside have not been limited to seeds. Various studies have examined the staining of the stigma surface, style canals, and pollen of Gladiolus (2,12-15). Similar to the staining of Gladiolus style canals and their mucilaginous contents was the staining of the secretory canals in the leaves and stems of ivy (Hedera helix). Ivy leaf and stem phloem tissue also stained weakly as did the phloem cell membranes of the petioles and leaves of Zantedeschia and Alocasia species (2). Sections of whole pollen grains stained at the periphery of the cytoplasm (especially spherical vesicles located in the inner layer of the intine). Reminiscent of the Gladiolus pollen staining was the specificity of the staining for the microspore perine of water ferns (Marsileaceae species); it is interesting that only the microspores (especially at their germinal sites) and not the megaspores contained the β-lectins and that here they were tightly bound (not elutable by water) and persisted after the onset of germination (16,17). In keeping with the β-GLU staining of the leaf parenchyma cell membranes of Zantedeschia and Alocasia species (2) was the discovery of β-lectins in the protoplast plasmalemmae of several species (9,18,19). At least, the presence of the β-lectins was strongly implied by the ability of β-GLU to stain and agglutinate protoplasts in a manner analogous to the erythrocyte or protoplast (20) agglutination induced by classical lectins such as ricin and con A.

The unifying factor for most of these observations seems to be that β-lectins are present in and around cell boundaries. Informative as this observation may prove to be, by itself it does not explain any function which the β-lectins may be serving in the cells' physiology.

CHARACTERIZATION

SPECIFICITY

As was noted in the Introduction, the β-lectins are defined by and named for their ability to bond specifically to a class of glycosylphenylazo dyes, the Yariv β-D-glycopyranosides. Subsequent studies (13,18,19,21,22) have further elucidated the Yariv compound stereochemical requirements for β-lectin bonding. These studies indicate that the bonding complement of a β-lectin must be a phenylglycopyranoside with the glycopyranosyl residue having a β-D or α-L linkage configuration at C1 and a free hydroxyl group in *trans* orientation to the anomeric linkage at C2. Furthermore, the phenyl group cannot be substituted at the *meta* position.

Jermyn (22) isolated a flavonol glycoside (myricetin 3-β-D-glucopyranoside) from the ethanolic extract of *Dryandra praemorsa* flowers that inhibited the precipitation of β-lectin by β-GLU (presumably by preferentially occupying the "active site" of the bond). He has pointed out the similarities in bonding ability and structure of the 3-glycosylated flavonols to the β-D-glycopyranosyl Yariv compounds and proposed that the former may be natural complements of the β-lectins.

The stoichiometry of the β-lectin β-GLY reaction has not been worked out due to the solution behavior of the Yariv compounds. For example, the Yariv glucoside has been found (23) to aggregate strongly in solution, forming "association polymers" of at least 40-50 molecules. The problem is further compounded by the current general ignorance concerning the structure of (and particularly the nature and number of

bonding sites within) a β-lectin. Despite these gaps in the understanding of the β-lectin/β-GLY interaction, the isolation of β-lectins by precipitation with the Yariv β-D-glycopyranosides is widely practiced. In fact, it is the only means, in contrast to other methods employed (11,24-28), by which the β-lectins can be uniquely isolated.

MOLECULAR WEIGHT

The molecular weight distributions of the β-lectins resemble those of other arabinogalactans (29) in the overall magnitudes and polydispersity observed. Ultracentrifugation methods have yielded weight average molecular weights for β-lectins of 126,000 [*Brassica napus* seeds (1)], 143,000 [*Acacia decurrens* seeds (30)], 313,000 [*Zantedeschia aethiopica* petioles (30)], and 275,000 [*Gladiolus gandavensis* style canal (31)]. Gel chromatography studies using Sepharose 4B columns have estimated β-lectin molecular weights of less than 500,000 [*Brassica napus* seeds (1)], 270,000 [*Lolium multiflorum* endosperm cultured cells (11)], 260,000 [*Gladiolus gandavensis* style canal (31)], and 224,000 [*Nicotiana tabacum* suspension-cultured cells (32)]. Using amino acid and carbohydrate analyses, the molecular weight of the β-lectin from *Phaseolus vulgaris* hypocotyl tissue was estimated to be 140,000 (33).

In light of the findings of this thesis, it is interesting that the molecular weights of β-lectins from dry seeds seem to be considerably less than those of secretory β-lectins (those from style canals and suspension-cultured cells). However, the accuracy of all of these values is open to question, since Woods has noted (30) the hazards of putting too literal an interpretation on the molecular weight values obtained by gel chromatography or ultracentrifugation. The former method is prone to error due to the difficulty of obtaining satisfactory calibrating materials with similar branched structures and known molecular weights; in the case of the β-lectins, where neither the detailed carbohydrate structure nor the molecular weight is known, nonempirically grounded assumptions must be made in choosing standards.

Similarly, changes in the carbohydrate moiety of these proteoglycans could cause large changes in the molecular weight values as determined by ultracentrifugation methods; i.e., changes in the composition, number, and cross-linking of carbohydrate sidechains could cause dramatic changes in charge density, partial specific volume, frictional coefficients, etc., which in turn affect the solution properties and behavior of the particles during a determination. In the absence of detailed structural information or molecular weight determinations by other methods, the directly determined sedimentation coefficient of the β-lectins may be a better comparative quantity than the calculated molecular weight. Unfortunately, the sedimentation coefficients of the β-lectins have not been customarily reported. The three reported values for the sedimentation coefficients of β-lectins are for Glycine max seed, 5.20S (7); Nicotiana tabacum suspension-cultured cells, 5.07S (32); and Pseudotsuga menziesii hypocotyl callus, 7.03S (34).

ELECTROPHORETIC TECHNIQUES

Reports on the electrophoretic mobility of β-lectins from various sources indicate that the arabinogalactan-proteins generally tend to consist of a continuous range of negatively charged components. The single, diffuse band generally observed after electrophoresis of a β-lectin sample is in keeping with the reported polydispersity of these compounds' molecular weight distributions.

Cellulose-acetate strip electrophoresis has been carried out on a number of different β-lectins. Jermyn and Yeow (1), using the Yariv glucoside as a stain, observed a relatively diffuse band spread out toward the anode for three purified angiosperm β-lectins. Employing the same stain but with a slightly more alkaline buffer (pH 8.8 instead of pH 7.0), the lectins from Gladiolus gandavensis styles gave a single, diffuse band which migrated slightly toward the cathode (27). Under identical conditions, the stigma surface β-lectin from Gladiolus gandavensis was

found to behave similarly to the style material (15,35). Gleeson and Clarke's further investigation of β-lectins from anthophytic sexual and somatic tissues revealed a somewhat greater charge diversity (36); the stigma and style β-lectins of Lilium longiflorum gave a single diffuse band as did those of Gladiolus under identical electrophoretic conditions, but the Lilium lectins possessed a slight negative charge in contrast to the Gladiolus β-lectins' slight positive charge. Still greater diversity was noted in the β-lectins from Gladiolus somatic tissues (leaf and petal), which gave three bands on electrophoresis: a minor component which migrated to the anode, a major component which migrated slightly toward the anode, and a major component which migrated slightly toward the cathode.

The only reported use of preparative electrophoresis on β-lectins also demonstrated the existence of charge diversity within a sample (37). In this report Gleeson and Jermyn, using a cellulose-acetate gel in barbitone buffer at pH 8.9, were able to fractionate the β-lectins from Alocasia macrorrhizos petioles into two components.

Polyacrylamide disc gel electrophoresis on the β-lectins has been reported twice, with results similar to those from cellulose-acetate strip electrophoresis. Bobalek and Johnson noted the ampholytic nature of β-lectins from three species of gymnosperm seeds (34). A single diffuse band (identified as glycoprotein β-lectin by its staining with PAS, Coomassie Blue, and β-GLU) was observed at a short distance from the samples' point of loading in both cationic and anionic electrophoresis trials. Under similar conditions, Akiyama and Kato also noted a slight mobility for the β-lectin isolated from Nicotiana tabacum suspension-cultured cells (32). The single, diffuse band they observed stained with both thymol-sulfur acid (for carbohydrate) and Yariv glucoside.

A first step toward quantitatively describing the charge character of the β-lectins was made by the use of an isoelectrofocusing column (33). In this

determination, β-lectin isolated from Phaseolus vulgaris hypocotyl tissue was loaded into the column and electrofocused. After three days the negatively charged β-lectin had found its isoelectric point at pI 2.3. To date there has been no report of analytical (flat-bed) electrofocusing of the β-lectins, which would be the next logical step in their quantitative charge characterization.

PROTEIN MOIETY

Weight Percent Protein

The magnitude of the protein moiety of the β-lectins (on a weight percent basis) appears to be a variable quantity. This is especially noticeable for the β-lectins from dry seed with a range from the 0.5% (1) for the gymnosperm conifer Sequoia sempervirens (redwood) to a maximum of 52% (2) for the angiosperm dicot Phaseolus lunatus (lima bean). However, most dry seeds examined have values which fall within a narrower range than this, as with Jermyn's (1) original survey (3-8%), the extension (2) of this survey (4-32%), and for the extensive sampling (38) from the Australian Acacia species (6-33%).

The β-lectins from nonseed sources tend to have a smaller magnitude and narrower range for their weight percent protein than those from dry seeds. The β-lectins isolated from the leaves of various species of higher and lower plants had a range of 3-8% protein (2). Other examples supporting this observation are the β-lectins from Phaseolus vulgaris (33) hypocotyl tissue (10%), from Gladiolus gandavensis (27) stigma surfaces (3%), and from suspension-cultured cells of Lolium multiflorum (11) endosperm (5-7%), and (32) Nicotiana tabacum (5%).

Clarke has noted the limitations of the comparative method (2). Single determinations of β-lectin samples from different species and tissue types have revealed that the protein moiety of the β-lectins is a variable quantity. Unfortunately,

without statistical sampling of β-lectins from a given source, it cannot be determined whether the observed variability is source dependent or merely due to random variation between individual samples of a single population.

Amino Acid Composition and Primary Structure

The similarity of the amino acid compositions of various β-lectins has been remarked since their discovery. In that publication (1) the β-lectin amino acid compositions from the dry seeds of seventeen spermatophytic species were compared. On the basis of amino acid residue mole percent of the total protein, five residues of comparable magnitude (glutamate, glycine, serine, aspartate, and alanine) comprised about half the protein, with variable amounts of hydroxyproline (0.9-16.5%). Subsequent amino acid analyses on β-lectins isolated from diverse sources sustain these observations, with the nineteen residues found having similar magnitudes from sample to sample (2,11,32,33).

The current failure to achieve the second step in determining the primary structure of a protein, the sequencing analysis, makes the information gained in the first step (the amino acid analysis) all the more important. Sequencing efforts have been frustrated by the multiple occurrence of covalent bonds between sugars and constituent amino acids along the protein's polypeptide backbone. To perform a traditional sequencing analysis, the protein would first have to be completely stripped free of carbohydrate. Such a task is difficult to accomplish, since mild methods of deglycosylation do not strip off all the sugars and strong methods decompose the polypeptide itself.

Because of the sequencing impasse, further structural studies of the β-lectin protein moiety have had to use the best compromise methods available, chemical and enzymic degradation (1,38-40) and N-terminal determinations (5) coupled with the limited sequence information obtainable through partial deglycosylation (41). These

studies indicate that the β-lectins tend to be glycosylated near the N-terminal of their polypeptide backbone. For the angiosperm β-lectins studied, degradation appears to proceed from the C-terminal end of the polypeptide with the total loss of such minor residues as cysteine and preferential retention of hydroxyproline. When the β-lectins from six angiosperm species were degraded, the peptide cores which resulted (apparently shielded from further degradation by the prosthetic carbohydrate groups) exhibited homologies in amino acid composition (39). As with the weight percent protein, in the absence of a complete sequence determination the degree of homology between the protein moieties of various β-lectins (regardless of whether the peptide core or undegraded protein is chosen as a basis of comparison) cannot be decided without further statistical studies.

Carbohydrate-Peptide Linkages

Because of the presence of hydroxyproline linkages in other arabinogalactan-proteins (29,41-43) and the preferential concentration of hydroxyproline in angiosperm β-lectin peptidecores (39,40), studies have been successfully directed toward demonstrating the existence of glycosyl-hydroxyproline linkages in the β-lectins. Jermyn (5) found his β-lectin sequencing attempts blocked at the next residue beyond the N-terminal (for angiosperm and gymnosperm species he found three N-terminals in the proportions of 2:1:1 for glycine:alanine:serine, suggesting a tetrameric structure for the β-lectins' protein moiety). Deglycosylation with anhydrous liquid hydrogen fluoride revealed the second residue to be almost invariably hydroxyproline. This indicated presence of a glycosyl-hydroxyproline linkage was confirmed in the β-lectin isolated from Phaseolus vulgaris hypocotyl tissue (44). However, the various hydroxyproline glycosides isolated accounted for only 39% of the glycosyl residues in the β-lectin. Some evidence was presented that the remaining glycosylpeptide linkages may be through serine, threonine, and asparagine, but the proportions and nature of such peptidyl glycosides were not determined.

CARBOHYDRATE MOIETY

Weight Percent Carbohydrate

β-lectins, as a good first approximation, can generally be considered as heteropolysaccharides. In fact, historically (7) the first three representatives of this class of natural products were identified as "seed polysaccharides" on the basis of their carbohydrate determinations (on a dry weight basis, the mean carbohydrate content was found to be about 80%). Later surveys (1,2) showed that a minor protein component existed, not as an impurity in the sample preparation, but rather as an integral part of the polymer. It was demonstrated that the entire weight of a sample could be accounted for by the carbohydrate and protein components (1,14) and that no other components were detectable (15). Hence the ranges of the weight percent protein given above are the complements of the ranges of the weight percent carbohydrate for the β-lectins. That is, the carbohydrate compositional extremes may range from 48% (lima bean) to 99.5% (redwood), with the usual range being 70-95% (dry seeds) or 90-98% (nonseed tissues). Because of this preponderance of carbohydrate, containing (as was later revealed) serially repeating units, the β-lectins are sometimes referred to as proteoglycans rather than glycoproteins (45).

Monosaccharide Composition

The β-lectins are essentially arabinogalactans. At least, the most abundant distinct monomers are galactose and arabinose, and it is not surprising that the original characterization of these "seed polysaccharides" failed to note the presence of small amounts of protein and minor sugars (7). On a mole percent basis of the total analyzed carbohydrate, the minimum reported sum to date of the galactose and arabinose monomers is 83% [for the β-lectins isolated from gladiolus styles, 82.7% (27) and kidney bean hypocotyls, 82.9% (33)]. Generally, the sum of the galactose and arabinose monomers comprises 87-95% of the analyzed carbohydrate; however, there

have been instances where they account for all of the carbohydrate present (1,2,11,24,31,36). In most cases galactose is approximately twice as abundant as arabinose, but the galactose to arabinose (G/A) ratio varies extensively. The minimum G/A value reported is for the β-lectin isolated from tobacco suspension-cultured cells, 0.90 (32) and the maximum for that from gladiolus style mucilage, 6.09 (31). Two different descriptive studies on β-lectins isolated from a variety of species and tissue types found G/A ranges of 1.24-2.78 (2) and 1.26-2.79 (11). These latter, lower values are more representative of the β-lectins and distinguish them from other arabinogalactans where G/A ratios of up to 14-16 have been found (29).

Neutral sugar analyses have detected up to seven different monosaccharides in the carbohydrate moiety of various β-lectins (2,11,14,15,27,31-33,36). In decreasing order of their frequency of occurrence and the magnitudes of their ranges (expressed as mole percent of the total analyzed carbohydrate), these neutral sugars are galactose (41.5-85.9%), arabinose (10.9-45.8%), glucose (0.0-17.3%), mannose (0.0-8.1%), rhamnose (0.0-6.3%), xylose (0.0-11.2%), and fucose (trace amounts).

In addition to the neutral sugars, small amounts of hexosamines (1,2,32,33) and uronic acids (1,32,33) have occasionally been found. These include glucosamine (0.0-0.2%), galactosamine (0.0-0.1%), β-D-glucuronic acid (0.0-11.5%), and mannuronic acid (0.0-9.4%). Although glucosamine is generally present, the uronic acid components are commonly absent or present in very small amounts (1,11).

Quantification of these β-lectin minor sugar components (the neutral sugars excluding galactose and arabinose, the hexosamines, and the uronic acids) has been hampered by three factors. First, they are present in such small amounts that a large initial sample is needed to detect them at all. Second, technical difficulties abound (chromatographic procedures must be modified and nonchromatographic methods employed). Third, these components appear to be located on the periphery of the

polysaccharide (capping the side chains); as such they are preferentially lost during any degradation which may occur during the isolation and purification of the β-lectin.

Structure

Figure 1 depicts a proposed model for the partial structure of the carbohydrate moiety of a β-lectin based upon published studies (11,31,35,32). Since these studies were conducted on a variety of β-lectins from different sources (species and tissues), it is quite possible that the basic features of the model are common to all β-lectins. The basic threefold features of this model are the same as those proposed for the "Aspinall Type II" arabinogalactans, a class of natural products found in all parts of higher and lower plants including the larch arabinogalactans and gum arabic (29,46). These three features are first, a galactan main chain composed of D-galactopyranose residues joined by β1→3 linkages; second, branched galactans composed of D-galactopyranose residues joined to one another and the main chain by β1→6 linkages; and third, arabinose-dominated side chain decoration of the branch β1→6 galactans. In its simplest form this last feature is represented by L-arabinofuranose residues joined by α1→3 linkages to the galactopyranose residues of the branch galactans (this arabinose substitution occurs along the length of the branch galactans and as caps on their terminals).

The three features of the foregoing model were deduced by the results obtained from methylation analyses on the β-lectins before and after they were subjected to partial acid hydrolyses (partial acid hydrolysis completely stripped off all of the arabinose, leaving a galactan-protein).

The configurations of the α-L-arabinofuranosides and β-D-galactopyranosides were determined by enzymic (31), immunological (47), optical rotation (47), lectin probe (14,15,25-27,36), and NMR spectroscopic (32) techniques. The last cited study also demonstrated the presence of D-glucuronic acid as a terminal residue of the β1→6

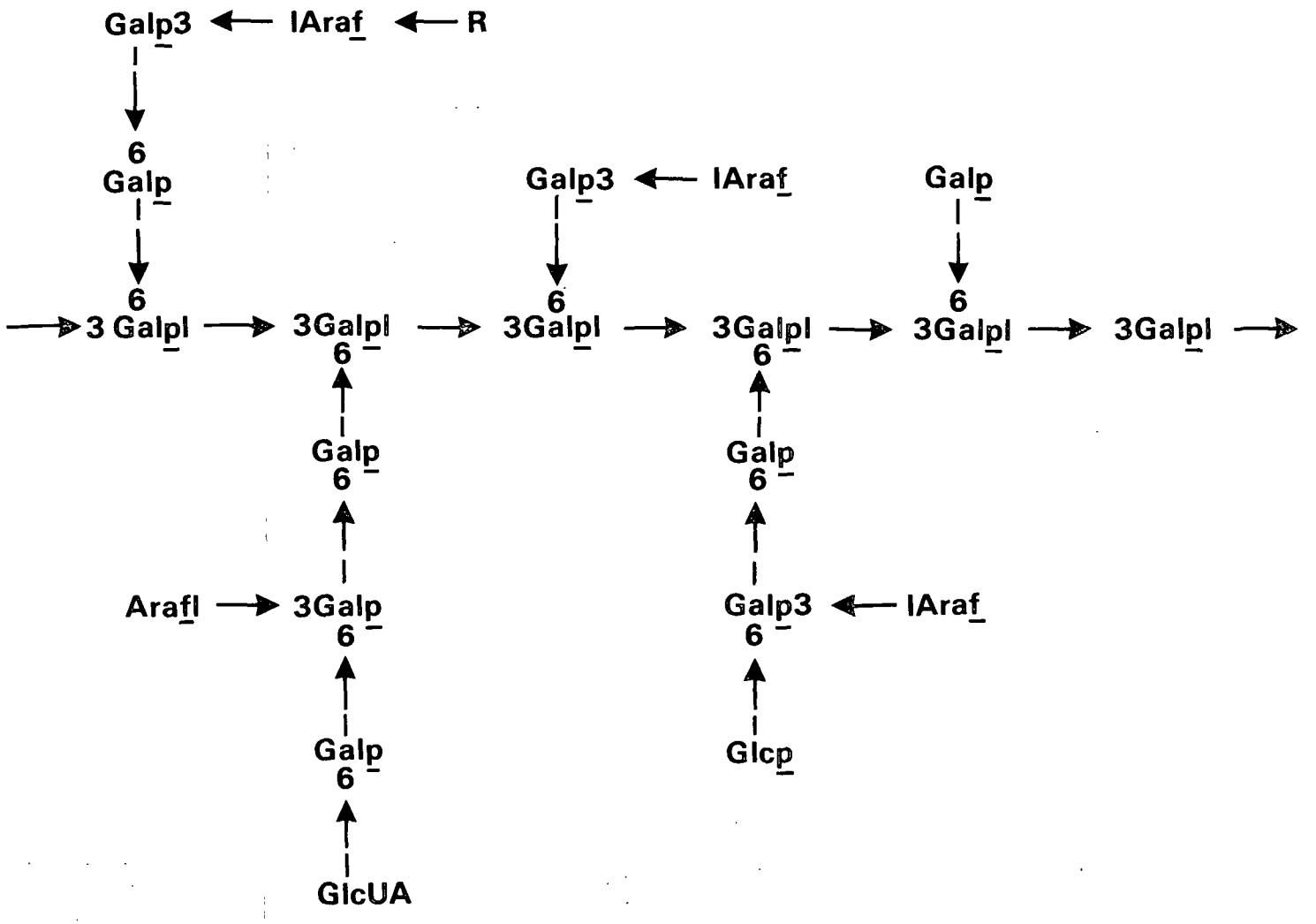


Figure 1. Tentative partial structure for the carbohydrate moiety of a β-lectin (R = an oligosaccharide, see text). The configuration of the pyranosyl groups is β-D; that of the furanosyl groups is α-L.

galactan branch chain (in a β-lectin isolated from suspension-cultured tobacco cells). Also, terminal D-glucopyranosyl residues have been found by methylation analysis (31) in the gladiolus style β-lectin.

The model of Fig. 1 also assigns a tentative position for other β-lectin minor sugars within oligosaccharide side chains attached to the α-L-arabinofuranosyl residues of the β1→6 galactan branch chains (by analogy with the structure of gum arabic). However, confirmation of this structural feature has not yet been reported.

BIOSYNTHESIS

Some β-lectins appear to be secretory glycoproteins. They have been detected cytologically concentrated between cells (10), in the mucilage of canals lined by secretory cells (2), and in the extracellular suspension-culture medium of cells from ryegrass (11), sweet cherry (48), sycamore (5), loblolly pine (34), kidney bean (33), and tobacco (32). Since they have also been detected in the cytoplasm (11,33), plasmalemma (18,19), and cell wall (33), it seems that the general pathway of glycoprotein biosynthesis has been followed. Sequentially this involves synthesis of the protein component on the rough (ribosomal) endoplasmic reticulum with subsequent enzymic hydroxylation of proline, glycosylation at the Golgi apparatus, transport to the cell membrane (possibly concurrent with further extension of the polysaccharide chains), and secretion from the cell (29,49).

Since there have been only two groups who have begun to look at the biosynthesis of the β-lectins, one in the Netherlands (33) and the other in Australia (50-54), details concerning any of the steps in the foregoing anabolic sequence are not known. However, certain aspects of β-lectin biosynthesis are beginning to emerge. Thus, both groups have confirmed the enzymic hydroxylation of proline as a post-translational event (52,54,33), a feature which the β-lectins share with extensin

and the collagens (29). In addition to this, Fincher et al. have shown that uridine diphosphate galactose (UDP-galactose) is a precursor of the galactan chain of the β-lectin and that batteries of anabolic glycosyl-specific enzymes are associated with the cell membrane. With further work, the details of β-lectin assembly and transport may prove indispensable for arriving at an understanding of these molecules' in vivo function.

FUNCTIONAL HYPOTHESES

There has been a great deal of speculation on the possible role of the β-lectins in plant physiology, particularly in view of their widespread occurrence throughout the plant kingdom. Clarke (55,29) has summarized a number of these β-lectin functional hypothese, which include:

(1) Plant defense [against fungal infection (56-58), bark rupture (gummosis), and extreme cold].

(2) Adhesion [of pectic substances and in the middle lamella, of pollen to stigma (pollen capture), of suspension-cultured cells to one another (cell clump diameter seems to be proportional to the β-lectin concentration of the medium) (59)].

(3) Nutrition [β-lectins in the style canal mucilage of gladiolus are thought to provide carbohydrate precursors for the growing pollen tube cell wall].

(4) Recognition phenomena.

The last possibility is the most intriguing of all, since it attempts to relate the function of the β-lectins to informational glycoproteins (60,61). Informational glycoproteins are molecules possessing carbohydrate residues arranged in a spatially well-defined, fixed form which bond to a complement (or fail to bond) and thereby

initiate a given event. Informational glycoproteins are known to occur across the spectrum of life forms from virus (the attachment of the parasite to the host cell's surface) to prokaryotes (the differentiation of the cellular slime molds) to algae (the fertilization phenomena of Fucus serratus) to sponges and embryonic chicks (histotypic aggregation phenomena) and so on up the scale to mammals (the uptake of sialoglycoproteins by the liver).

Given this abundance of functions across such diverse life forms, it would be surprising not to find such informational molecules present throughout the plant kingdom. In fact, such a carbohydrate-recognition event initiates pollination; pollination was inhibited in gladiolus (12) by the removal of β-lectin from the stigma surface and in cabbage (62) by the addition of a stigma-derived glycoprotein (compositionally not dissimilar to a β-lectin) to the pollen. The other striking example of a recognition event (cell-cell recognition) in plants is the stem-grafting experiment, which is successful only when the species are not too genetically diverse (63). Mediation of the tissue-junction event has not been shown to involve recognition of cell-surface carbohydrate determinants, although tissue-cultured cells have been shown to retain distinct histogenetic markers even after four subcultures (48). Only one set of comparative studies has been carried out on the β-lectins from the various tissues of a single species to examine the qualifications of these arabinogalactan-proteins as informational molecules. These anthophytic studies (35-36) indicated that there were slight differences in the carbohydrate compositions of β-lectins isolated from gladiolus tissues (stigma, style, leaf and petal) and lily (stigma and style). Although no null hypotheses and significance criteria were established, the results did not negate the possibility that the β-lectins were significantly different from one another.

PROPOSAL

THESIS OBJECTIVE

The preceding review of the literature on the β-lectins revealed that research on these mysterious compounds has been generally of a qualitative and exploratory nature. It is unlikely that major advances in understanding the β-lectins can be made without more detailed, quantitative studies. This thesis was undertaken as a first step in such a direction, with the following objective:

> Test for the presence of and estimate any variations in the β-lectins from two related species during early growth with the purpose of determining the potential of the molecules as developmental, taxonomic, and histogenetic markers.

The second part of this objective was contingent on an affirmative result for the first part. If the β-lectins were not present, they could not be characterized nor could they serve any function in the part of the plant's life in which they were absent. If the β-lectins were not structurally variable (did not significantly differ from one another in content or form) or varied in a random manner, they could not be used as markers (related to other events of plant physiology). If the molecules were markers, then it might be possible to form hypotheses to direct research toward defining their function in the plant.

APPROACH

The two species used were Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] and loblolly pine [Pinus taeda]. Both gymnosperms are conifers of the Pinaceae family and were thought to be closely enough related (64) to have permitted independent verification of any developmental trends that might occur while still allowing for the possibility of detectable taxonomic diversity (species-dependent differences).

The period of early growth was chosen as the one most likely to have revealed any developmental changes as the plant grew from an embryo to a young tree. The developmental states sampled were those of the dry seed (DS), stratified seed (SS, the cold, water-soaked seed at the onset of germination), cotyledon seedling (CT, the seedlings approximately two weeks after germination, before the primary needles have fully developed and opened), two-month-old seedlings (TM, after the primary needles have fully developed and opened), sapling (SP, one to four-year-old trees), and callus (CL, dedifferentiated cells produced as a wound response and propagated under laboratory tissue culture conditions). The two-month seedlings and saplings were separated into needles, stems, and roots to test for histogenetic (tissue-specific) differences. These six different developmental states are depicted schematically in Fig. 2.

The thesis objective was implemented by systematically addressing a pair of major null hypotheses. These were:

(1) There are no β-lectins in the tissues of a developing conifer [this null hypothesis was tested by examining the twenty tissue sources described above].

(2) There are no differences in the β-lectins of developing conifers [this null hypothesis was tested by characterizing the isolated β-lectins via the six parameters described below and comparing the respective means for statistically significant differences].

The research effort was directed at invalidating these hypotheses by finding, respectively, that the β-lectins were present in the various conifer tissues and that they did differ significantly from each other depending on their tissue of origin. Absence of the β-lectins from a given tissue would have implied that they did not serve any role therein. Identity of all parameters in all samples would have

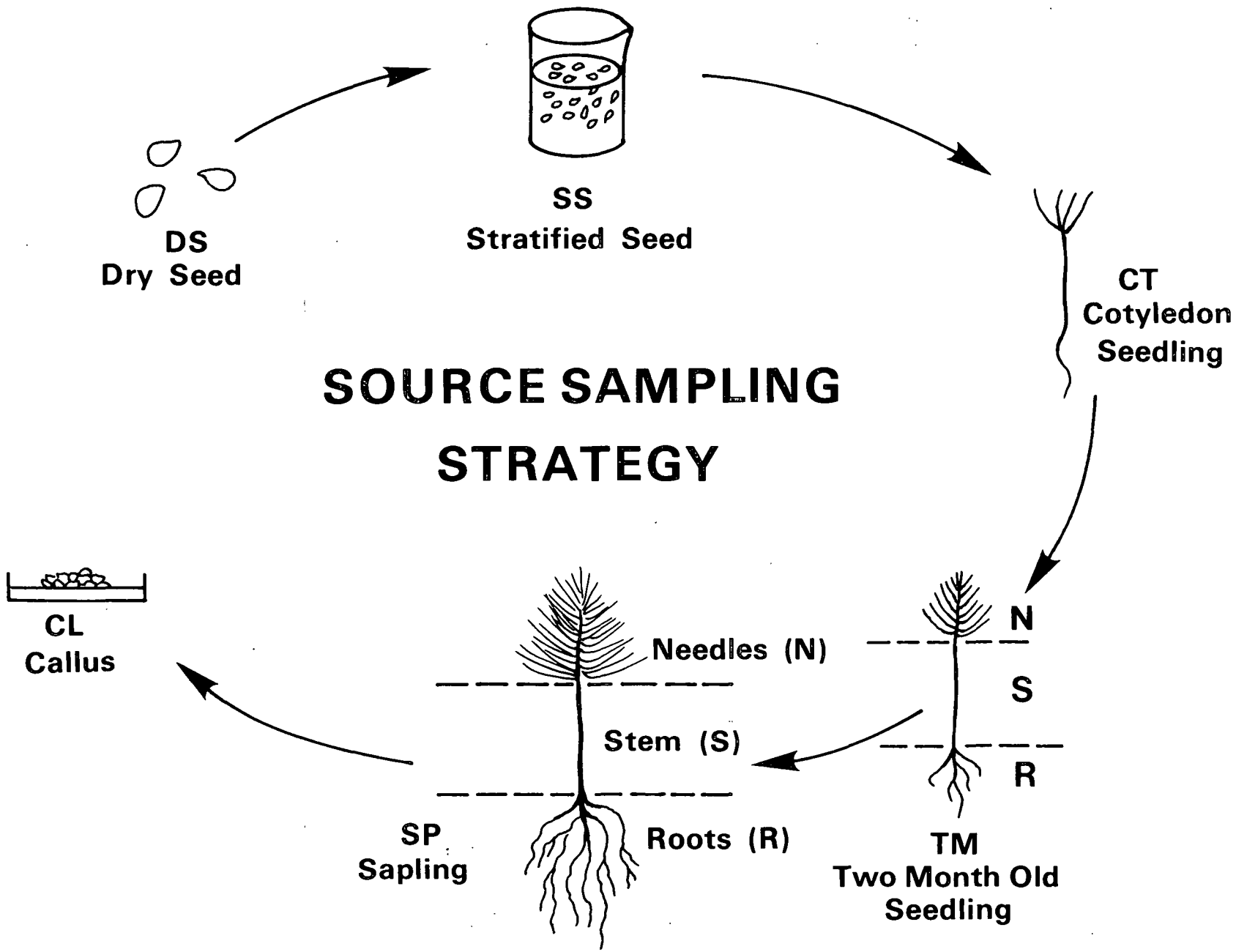


Figure 2. Plant materials from which β-lectins were extracted.

implied that the β-lectins are invariable in structure and cannot serve as biochemical markers.

The presence of the β-lectin in a sample of homogenized tissue was determined by a double diffusion precipitin test against Yariv glucoside. Upon confirmation of the presence of the β-lectin, the Yariv glucoside was used to precipitate the compound in bulk from its tissue extract. After purification of the complex, the β-lectin was separated from the Yariv glucoside and prepared for analysis.

Six parameters were selected to characterize the β-lectins, four of them of a quantitative and two of them of a more qualitative nature. The quantitative measures were obtained via an amino acid analysis and weight percent protein determination (to monitor any qualitative and quantitative changes in the protein moiety), a carbohydrate analysis (to monitor any compositional changes in the neutral sugars of the carbohydrate moiety), and a sedimentation-velocity determination (to monitor the sedimentation coefficient, a directly determined quantity proportional to the molecular weight of the molecule). The qualitative measures were obtained via electrofocusing (separation of the charged components of the molecule by their isoelectric points) and Fourier transform infrared spectroscopy (estimation of the quantity and quality of a molecule's functional groups by their idiosyncratic patterns of infrared radiation absorption).

Three to six separate isolations of the β-lectins from each state were conducted so that a two-way analysis of variance could be run on each parameter and a statistically significant measure of the source-dependent differences in the means obtained (see Results). The results are discussed in terms of the degree of the parameter's similarity in each species, its difference between species, its intraspecies differences among the plant parts, and its intraspecies differences among the developmental states.

MATERIALS AND METHODS

YARIV GLUCOSIDE

The artificial carbohydrate antigen [1,3,5-tri-(p-β-D-glucosyloxyphenylazo)-2,4,6-trihydroxybenzene, or Yariv glucoside (β-GLU) with the structure as depicted in Fig. 3] was synthesized by coupling diazotized 4-aminophenyl glucoside with phloroglucinol (65) as shown in Fig. 4.

The p-aminophenyl-β-D-glucopyranoside was purchased from Calbiochem, San Diego, CA (A grade, m.p. 159.5-161°C). Phloroglucinol (1,3,5-trihydroxybenzene) was purchased from Eastman Organic Chemicals, Rochester, NY, and its purity was determined by a melting point determination [found: m.p. 218-219°C compared with the 219°C reported in the literature for its anhydrous form (66)].

The reaction was run in a 150-mL beaker containing a clean Teflon-coated one-half-inch long magnet (stirring bar). The beaker was immersed in a glass ice bath on a magnetic stirrer. The reaction was followed with a Beckman pH meter fitted with a Markson microelectrode and thermometer.

To assure complete substitution of the phenolic core, a ratio of 3.1-3.5 millimoles of the amine to 1.0 millimole of phloroglucinol was employed. The amine was diazotized in 24 mL of 0.5N HCl at 0-3°C by the rapid addition of 0.828 g $NaNO_2$ (sodium nitrite) for the *in situ* production of HONO (nitrous acid). The pH of this diazonium salt solution was adjusted to 6-7 with 0.5N NaOH, then to pH 8.9 with 0.1N NaOH. The phloroglucinol in a small amount of distilled water was gently stirred into the diazonium salt solution, which immediately turned a dark purple color. The pH of the reaction mixture was maintained in the range 8.4-9.0 by addition of 0.1N NaOH. The ice bath was replenished during the early stage of the coupling to maintain a temperature of 2-12°C, which facilitated pH control. After about 2 hours the pH became stable, and the reaction mixture was dialyzed for 24 to 30 hours with 4 to

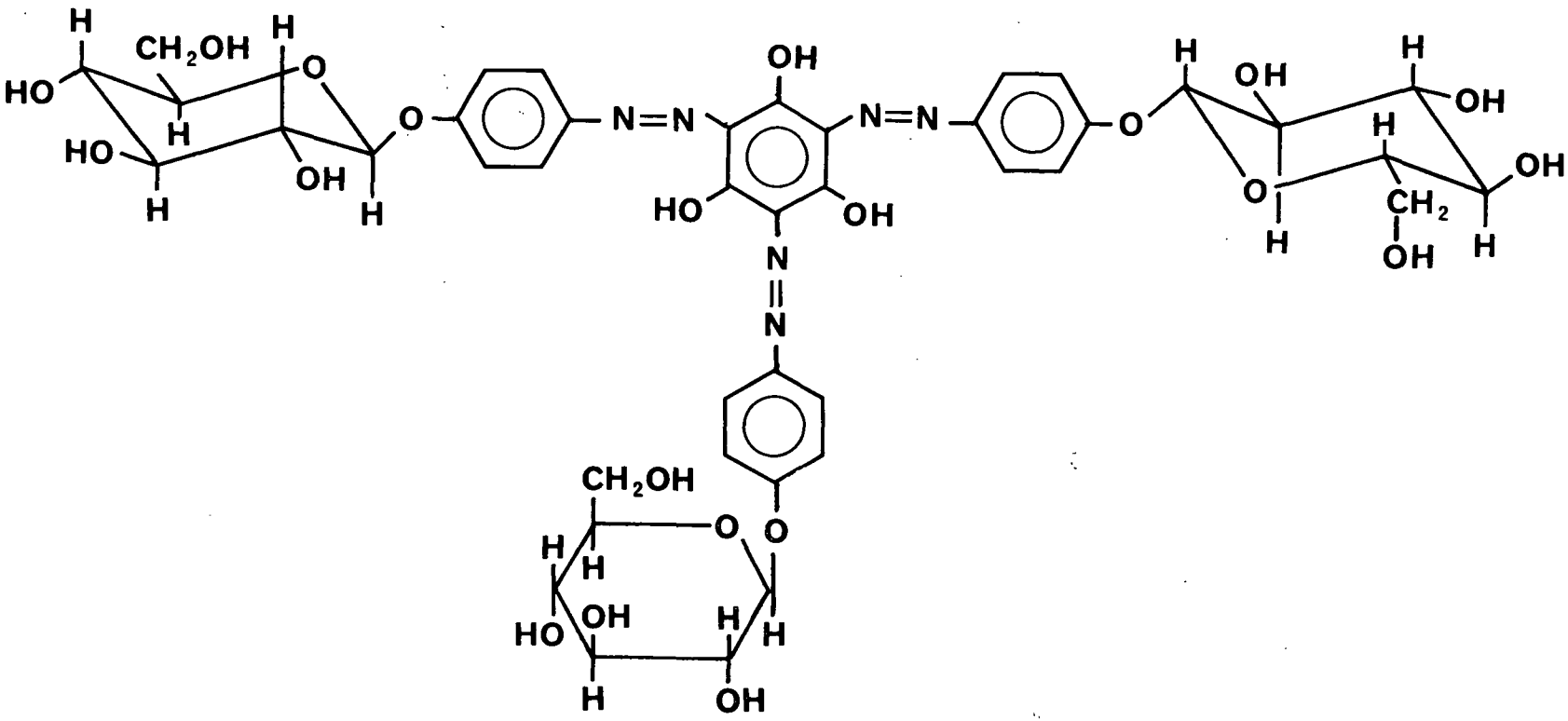


Figure 3. Structure of the Yariv glucoside (β-GLU): 1,3,5-tri-(p-β-D-glucopyranosyloxyphenylazo)-2,4,6-trihydroxybenzene.

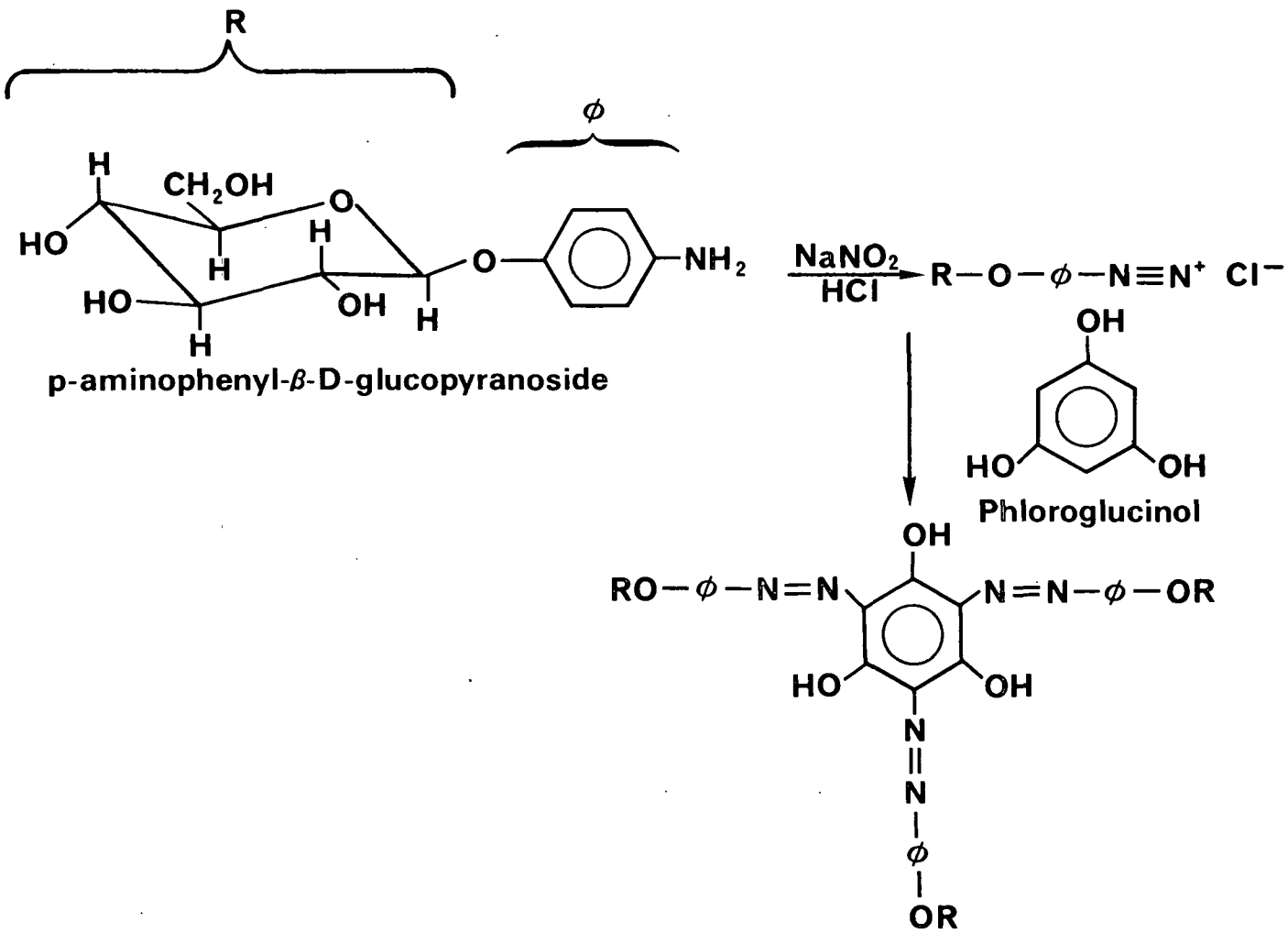


Figure 4. Synthesis of the Yariv glucoside, β-GLU: ∅ = the disubstituted phenyl group, C_6H_4, and R = the glucopyranosyl group, $C_6H_{11}O_5$.

5 changes of 80 volumes of distilled water, the last change being with double-distilled water. The Yariv glucoside was further purified by precipitation with methanol, refrigeration for 12 to 15 hours at 3-5°C, and collection of the precipitate by centrifugation. The precipitate was redissolved in a little double-distilled water and lyophilized.

The absorption spectrum of the lyophilizate was like that of the compound described by Yariv (65), with extrema at 398 nm (maximum), 450 nm (minimum), and 485 nm (maximum). As with the Yariv galactoside, there was no change in the absorption spectrum of the compound for conditions of acidic, neutral, and mildly alkaline pH. However, at pH 12 or greater, a single broad maximum was found at about 440 nm (Fig. 5). Conclusive identification of the synthesis product as the Yariv glucoside came by comparison of its behavior in precipitin tests and spectrophotometrically with that of a highly purified sample received from Dr. Michael Jermyn, used by him in a study on the Yariv dyes' aggregation in aqueous systems (23). In precipitin tests, the synthesis product gave lines of similar intensity and appearance against all plant materials which Jermyn's β-GLU reacted with; furthermore, in cross-well tests, the lines formed by the synthesis product merged smoothly with those formed by Jermyn's gift. Finally, the superimposability of the visible (Fig. 5) and infrared (Fig. 6) spectra of the two compounds confirmed their identity.

SPECTROPHOTOMETRY

Absorption spectra on the Yariv glucoside were run on a Perkin-Elmer Model 576 ST recording spectrophotometer from 330 to 580 nm at 20°C with distilled water as solvent in 1 cm (beam pathlength) cuvettes.

Infrared spectra were run on KBr (potassium bromide) pellets of the compounds on a Nicolet 7000 Series Fourier Transform Infrared Spectrophotometer from 4000 to 400 cm^{-1}.

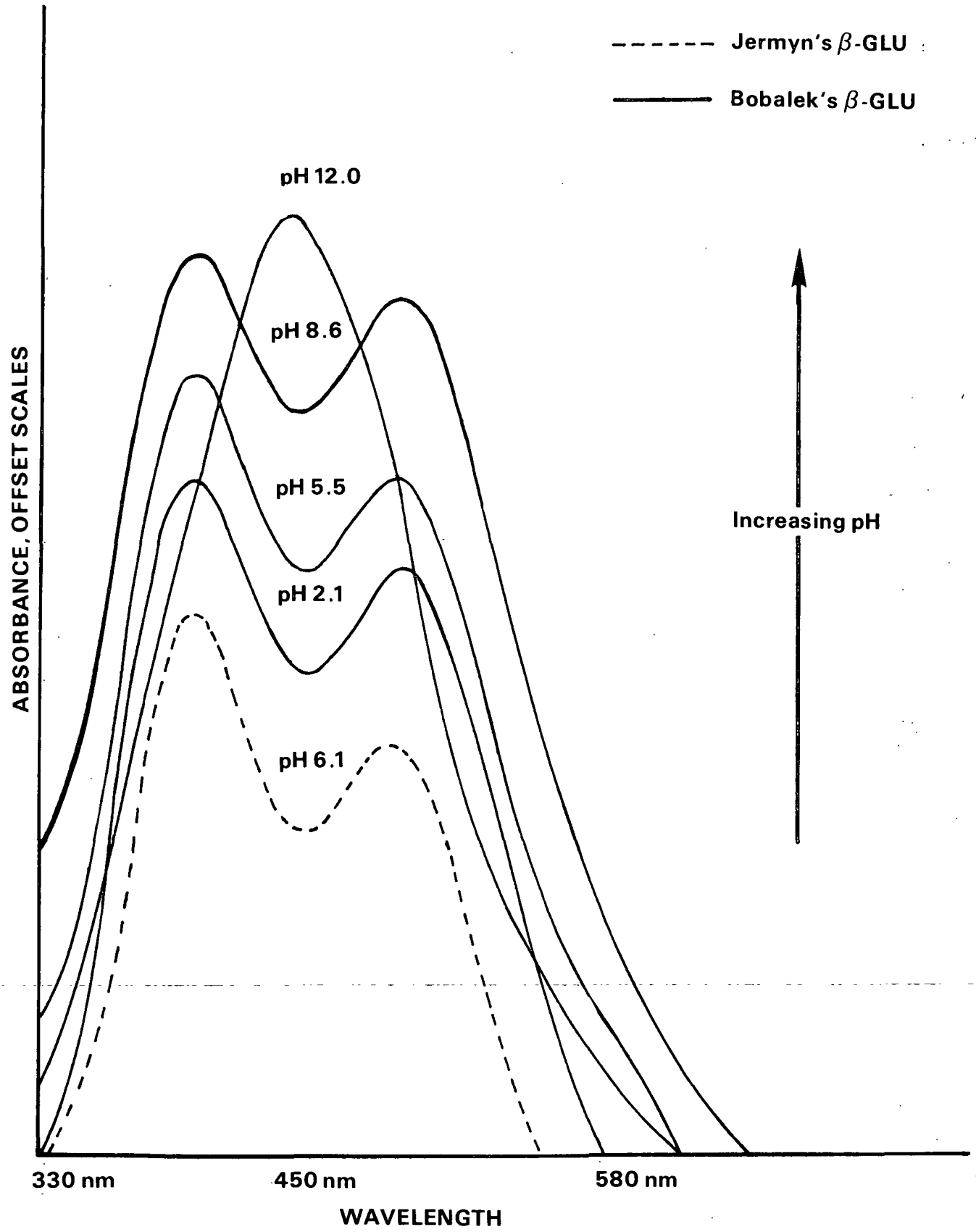


Figure 5. Change in the visible absorption spectrum with increasing pH for the Yariv glucoside (β-GLU). Scales have been offset for clarity.

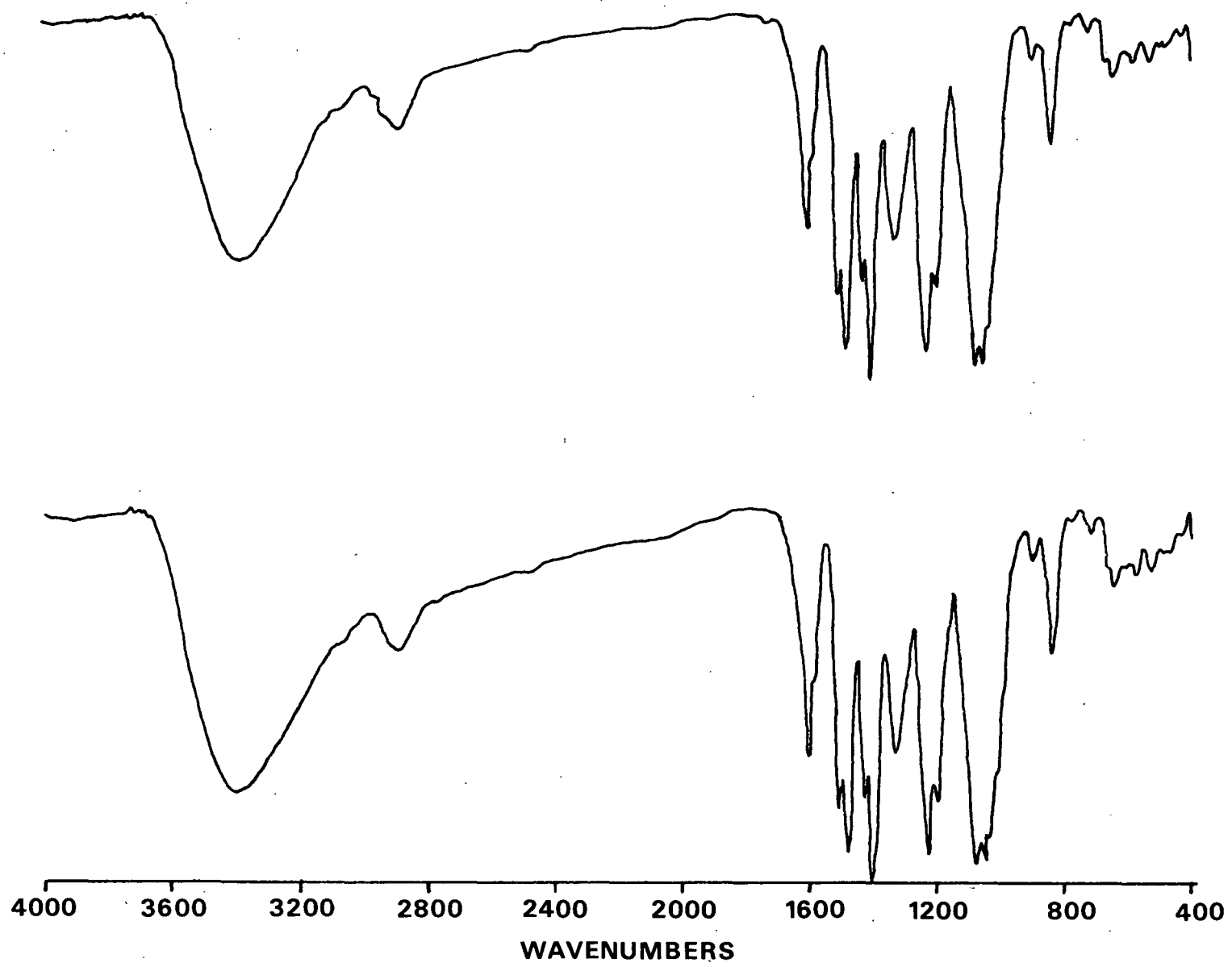


Figure 6. Comparison of Fourier Transform Infrared Transmittance Spectra for donated (top) versus synthesized (bottom) Yariv glucoside.

PRECIPITIN TESTS

The double diffusion tests were carried out similarly to those of Jermyn (1). Gel-diffusion plates were prepared by dissolution of 1% (w/v) agar in the standard extracting buffer, PBS [0.02M potassium phosphate buffer at pH 6.8-7.2 with 1.0% (w/v) NaCl and 0.01% (w/v) NaN_3], autoclaving, and pouring under a laminar air-flow cabinet into 5-cm-diameter Petri dishes (about 5 mL per dish); suction-uptake was used to plunge nine wells in a three-by-three matrix in the plates at a separation of 5 mm. Four of these wells were filled with a solution of 0.1% (w/v) β-GLU in distilled water (stored at 3-5°C when not in use). The remaining wells were filled with a PBS extract of the plant material to be tested. While alcoholic extraction normally preceded PBS extraction, sometimes during the survey portion of the study, the plant material (especially seeds) was not subjected to a preliminary alcoholic extraction to remove potential inhibitors of the precipitin reaction. Four to six hours after loading the wells, a positive test (for the presence of the β-lectin) was indicated by the appearance of a red precipitin line between the wells.

PLANT MATERIALS

Douglas-fir seeds were obtained from Brown Seed Co., Seedlot Number 579 [The Institute of Paper Chemistry (IPC) Seedlot Number 3223-19], collected in northwestern Washington at an elevation of 2000 to 2500 feet. The loblolly pine seeds were purchased from Herbst Brothers Seedsmen, Inc., Seedlot Number 6313A (IPC Seedlot Number 3223-20). The seeds were stored at -15°C until used.

Three samples (200 g, 400 g, and 400 g) were withdrawn from each of these seedlots and used as source material for the isolation of the dry seed (DS) β-lectins. Further portions of the two seedlots were stratified by submersion in tap water for 48 hours, drainage of excess water, and refrigeration of the seeds at 1-2°C for 60 days (67). Three samples of these soaked seeds (400 g each) were withdrawn as

source material for the stratified seed (SS) and the remainder used to seed 4 redwood flats per species, grown according to the greenhouse conditions described by Smits (68). After 24 days, the 9 to 11-cm-long cotyledon seedlings of two flats were harvested as source material for the cotyledon seedling (CT) β-lectins. The remaining two flats were harvested 64 days after planting, and the 14 to 16-cm-long seedlings were used as source material for the two-month-old seedling (TM) β-lectins.

Different seedlots were used to start the seedlings used as source material for the sapling (SP) β-lectins, although the conditions of stratification and growth were the same. The Douglas-fir seed was from Weyerhaeuser Company Seedlot 491-15-1 (IPC Seedlot Number 3223-10). This seedlot was collected at an elevation of 1500 feet from a point west of the Cascades near East Roseburg, Oregon. The loblolly pine seed was purchased from International Seed Company (IPC Seedlot Number 3223-1) and was harvested in Cullman County, Alabama. The Douglas-fir saplings were approximately 460 days old (1.3 years) when harvested. The loblolly pine saplings were older; about 1310 days (3.6 years) had elapsed from the time of sowing.

Douglas-fir bud tip callus (IPC Clone Number DF No. 9B) was initiated from a 32-month-old sapling and grown on a modified Murashige and Skoog medium (IPC Medium MS11, Appendix I) with 7 subcultures. Subculturing involved transferring fragments of the mother-tissue to fresh medium and was performed about once a month; the total time from callus initiation to harvest was 282 days. Fluorescent lamps provided lighting of 125-175 footcandles. The incubator automatically was set to cycle between 16 hours of light at 23°C and 8 hours of dark at 19°C. Each four-inch-diameter glass Petri dish (sealed with Parafilm) contained five clumps of friable dark-green callus.

Loblolly pine stem callus (IPC Clone Number LP-S14) was initiated from a three-month-old seedling and grown on another modified Murashige and Skoog medium (IPC

Medium-1, see Appendix I) with six subcultures. The total time from callus initiation to harvest was 203 days. The callus was grown on a cycle of 16 hours of light and 8 hours of dark at a constant temperature of 23°C. Again, four-inch diameter Petri dishes were loaded with five clumps of moist light-green callus and sealed with Parafilm. Loblolly pine callus and other coniferous sources of β-lectins used in this study are shown in Fig. 7.

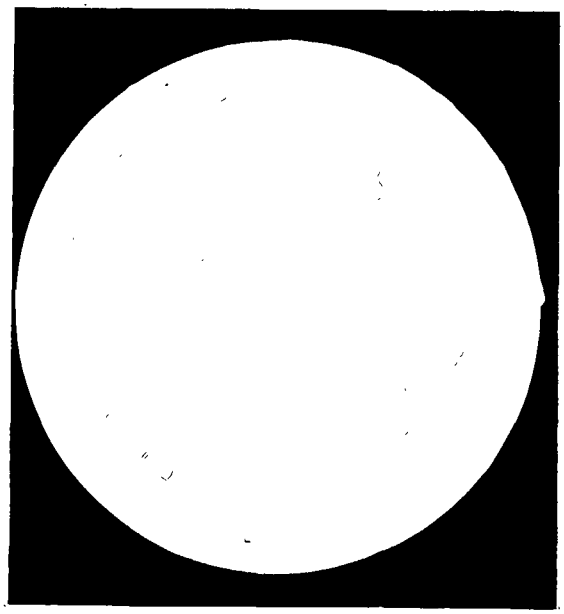
ISOLATION AND PURIFICATION OF THE β-LECTIN

The method used for the extraction of the β-lectins from plant material and their subsequent purification is essentially that of Jermyn (1) as modified by Clarke et al. (2). The method consists of a double extraction of the plant material followed by precipitation of the β-lectins via the Yariv glucoside (extreme left, Fig. 9). Due to the solubility of the complex in distilled water, it can be further purified by a series of reprecipitations. This general procedure for the isolation of the β-lectins and the purification of the complex is shown in Fig. 8.

The ethanolic extraction is required to remove inhibitors of the precipitation reaction between the β-lectins and the Yariv glucoside and to remove lipids which interfere with the clarification of the aqueous extracts. In every case except the sapling material (SP), comminution of the plant material was carried out in the presence of boiling (73-78°C) ethanol. A large stainless steel Waring Blendor was used to grind the dry seed, stratified seed, and the freshly gathered coarsely chopped two-month-old seedlings (separated into needles, stems and roots). The calli and cotyledon stage seedlings were ground with a porcelain mortar and pestle. The sapling material was of such size and extracted in such quantity that a Wiley mill was used to grind the freshly gathered, separated (needles, stems, and roots) portion. In this case only, due to the impracticality of using hot solvent in the mill, the ground material was extracted with boiling ethanol following grinding.

LEFT: Sampling material. Left to right: 1.3-year-old Douglas-fir, 1.6-year-old loblolly pine, and 3.6-year-old loblolly pine.

RIGHT: Loblolly pine sources. Left to right: seedlings at two months to two weeks (cotyledon stage) and dry seeds.



LEFT: Loblolly pine callus.

Figure 7. Coniferous sources of β-lectins.

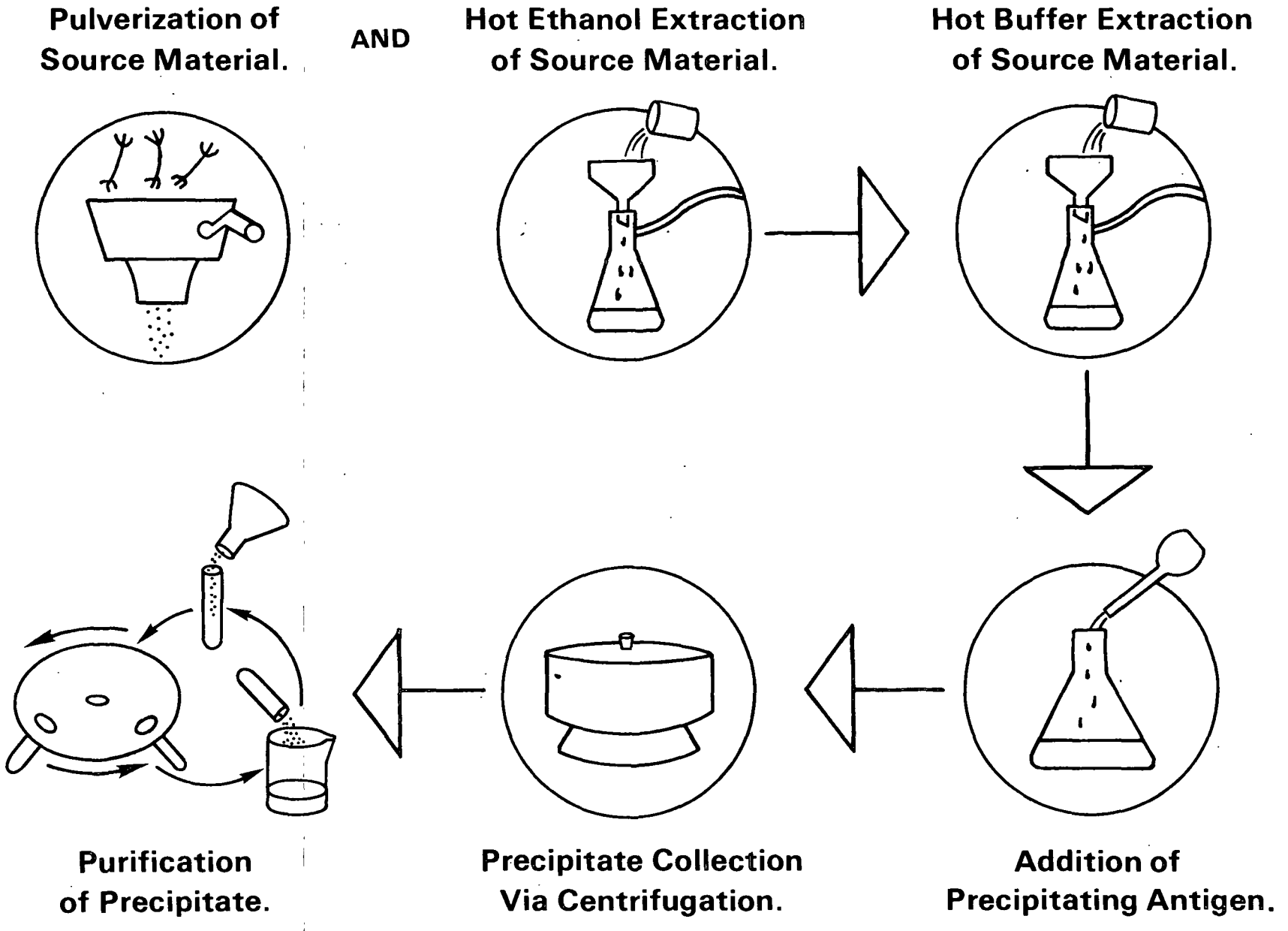


Figure 8. Isolation and purification of the complex.

The finely ground material in every case was transferred to a coarse sintered glass filter funnel, and boiling ethanol (absolute) was poured over the material until a clear filtrate was obtained.

After air-drying to constant weight, the powdered residue from the ethanolic extraction was extracted for 5 to 10 minutes with ten successive (v/w) portions of boiling (95-100°C) 0.02M potassium phosphate buffer, pH 7.0 ± 0.2, containing 1.0% (w/v) NaCl and 0.01% (w/v) NaN_3 (sodium azide). In the case of the Douglas-fir stratified seed extraction, the filtrate was further clarified by addition of acetic acid (to pH 4.8), centrifugation to collect the precipitated globulins, addition of 1.0N KOH (to pH 7.0), refrigeration for 2 hours to precipitate further impurities, and centrifugation. An excess of precipitating solution [0.1% (w/v) β-GLU in distilled water with 1.0% (w/v) NaCl and 0.01% (w/v) NaN_3] was added with stirring to this clarified supernatant, and directly to the clear filtrate for all other extracts. This ruby-colored solution was refrigerated 10 to 12 hours and the precipitate collected in conical glass tubes via a Dynac (Clay Adams) desktop centrifuge. The complex was purified by dispersal in distilled water, removal of the chalkish-gray insolubles by centrifugation, and reprecipitation of the complex by addition of 10% (v/v) sodium chloride. This sequence was repeated two to three more times until no further insolubles could be removed. Finally, the complex was washed twice with methanol (absolute) to remove the sodium chloride used in the reprecipitations.

The complex could be lyophilized and stored (middle Fig. 9) or immediately reduced with 10% (w/v) sodium dithionite solution (pH 5.0), prepared by dissolution of the reducing agent in distilled water immediately before use.

Removal of the Yariv compound by reductive disruption of the azo function in the molecule could be hastened by gentle warming of the orange solution. The resulting pale yellow solution was placed in cellulose dialyzer tubing with four changes of 80

volumes of distilled water over 24 to 30 hours, the last change being with double-distilled water. The dialyzed solution was passed through a 45-μm Millipore filter and the colorless, clear filtrate lyophilized. The lyophilizate was a feathery white compound which produced strong, razor-thin precipitin lines against the Yariv glucoside. This lyophilizate (extreme right, Fig. 9) was used in the ultracentrifugation determinations, carbohydrate analyses, isoelectric focusing trials, and Fourier transform infrared spectrophotometer scans.

Figure 9. Chemical sequence for β-lectin isolation. Left to right: β-GLU, β-GLU/β-lectin complex, purified β-lectin (all compounds are shown lyophilized).

AMINO ACID ANALYSES

Amino acid analyses were run on a Beckman Amino Acid Analyzer Model 119CL according to the method of Spitz (69).

Samples of the purified complex were placed in Kontes hydrolysis tubes (3 mg per tube) with 2.0 mL of constant boiling hydrochloric acid (5.7N HCl). The tubes were

evacuated, purged with nitrogen twice, sealed, and kept at 105°C for 20 hours. Next, 2.0 mL each of pH 2.2 citrate buffer and buffer neutralizer solution prepared according to Spitz (69) were pipetted into the hydrolysis tubes. The tubes were centrifuged in a Sorvall Clinical Centrifuge to remove humin from solution. The supernatant (3.0 mL) was pipetted into clean glass vials, and the pH was adjusted to 1.8-2.0 with saturated lithium hydroxide solution at ca. 20°C. The samples were frozen until they could be filtered and loaded onto the analyzer.

SEDIMENTATION-VELOCITY DETERMINATIONS

The sedimentation-velocity determinations were conducted on a Beckman Model E Ultracentrifuge. A single sector, synthetic boundary cell with quartz windows and Schlieren optics was used. The determinations were made on the purified β-lectins (5 mg/mL) in buffer solution [0.02M potassium phosphate, pH 7.0, containing 0.17M NaCl] at 25°C. Photographic glass plates were exposed at 2-minute intervals 6 to 9 times after the rotor had reached 56,000 rpm (r 6.5 cm). Sedimentation distances were read off the Schlieren patterns with a microcomparator and punched onto computer cards. The sedimentation coefficients were then calculated on an IBM 360 Computer using the SEDCO program.

CARBOHYDRATE ANALYSES

The neutral sugar composition of the carbohydrate moiety of the β-lectin for the different samples was determined by GLC (gas-liquid chromatography) analysis of alditol-acetates as prepared by the method of Borchardt and Piper (70).

About 3 mg of each sample of purified β-lectin was dried for 14 to 16 hours over P_2O_5 (phosphorus pentoxide) before being weighed on a semimicro analytical balance to 0.01 mg. Mineral acid (sulfuric acid) hydrolysis of the samples was followed by reduction of the monosaccharides to their corresponding alditol-acetates. Multiple

injections were made into a Packard Model 417 gas chromatograph connected to a Hewlett-Packard 3385A data system. The integrated peak areas were reported on a percent basis of the total neutral sugars present.

ISOELECTRIC FOCUSING

The purified β-lectins were electrofocused on polyacrylamide gels containing carrier ampholytes (LKB Ampholine PAG plates) with an LKB 2117 Multiphor System. The runs were conducted according to the manufacturer's recommended procedure (71).

The β-lectins were dissolved in 30% DMSO [dimethylsulfoxide being a better solvent for the AGPs than 1% glycine at a concentration of 10-13 mg/mL (130-200 μg/gel slot loaded)]. The gels were prefocused for one-half hour to establish a pH gradient from 3.0 to 9.5. An anode solution of 1M H_3PO_4 and cathode solution of 1M NaOH were employed. The β-lectin samples were applied to the surface of the gels with 1.0-cm filter paper wicks. Constant power (25 watts) was applied for half an hour, the filter wicks removed, the same wattage reapplied for another hour, and the pH gradient determined with a microelectrode.

The isoelectric points of the β-lectin components were located by staining of the electrofocused gels. Three stains were utilized: Coomassie Brilliant Blue for protein, PAS (periodic acid Schiff's reagent) for carbohydrate, and the Yariv glucoside for the β-lectins. The Coomassie Brilliant Blue was applied according to the LKB method (71). The PAS staining procedure followed was that of Kapitany and Zebrowski (72). The Yariv glucoside was applied as a 0.1% (w/v) solution in 0.1M NaCl and 0.01% w/v NaN_3. The gels were immersed in the Yariv glucoside solution for 12 to 14 hours and destained in 0.1M NaCl. All of the gels were photographed, and projected later for comparison with the notes and sketches made on them immediately after destaining.

COMPUTATION

The sedimentation coefficients were calculated on an IBM 360 Computer using the SEDCO program. The analyses of variance were computed on a Burroughs 6900 System using Utah State University's Statpac (statistical package). The particular software employed from Statpac was a two-way multiple regression analysis of variance [two-way analysis of variance with interaction, AOV(2)] capable of dealing with an unequal number of sample observations.

RESULTS

OCCURRENCE OF THE β-LECTINS

The β-lectins were found in every tissue source shown in Fig. 2. However, the precipitin tests of the plant materials differed from one another in two ways. First, certain tissues would not yield the characteristic red precipitin lines indicative of the presence of the β-lectins without a preliminary extraction with hot absolute ethanol. Thus, with loblolly pine, although the dry seeds, spent medium (the liquid nutrient in which suspension cells had been growing), and sapling stems all gave positive tests, the sapling roots gave only negative tests (no precipitin lines) until they were extracted with hot ethanol. Second, the intensity of the precipitin lines varied from tissue to tissue. For example with Douglas-fir, the alcohol-extracted sapling needles gave twice the intensity of lines as that from the alcohol-extracted stems or roots (in each case, 304 mg of the alcohol-extracted plant material was ground in 4.0 mL of PBS solution). Another example of the varying intensity of the precipitin lines is shown in Fig. 10, where no lines were detected for the crushed seed coat from Pinus edulus, but where weak lines were detected for its excised endosperm tissue and strong lines for its excised embryos (all preparations were tested at the same concentration). Further observations were also made concerning the occurrence of the β-lectins throughout the subkingdom Embryophyta (especially among the Coniferales) and their absence in all tested representatives of the subkingdom Thallophyta (73).

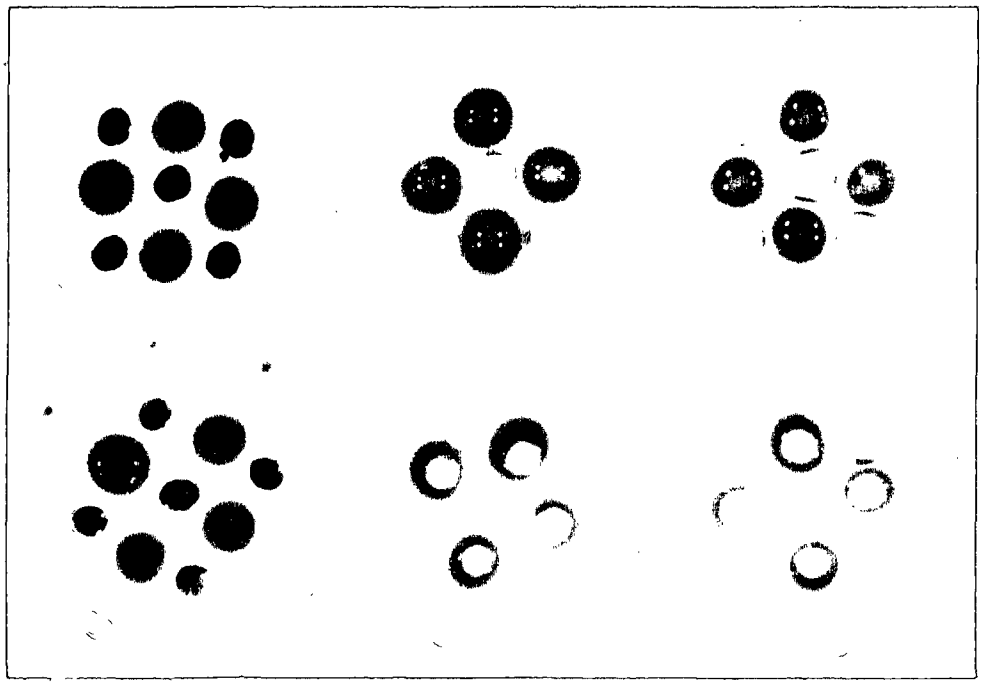


Figure 10. Variable line intensity of the precipitin test. From left to right, Pinus edulis seed coats, endosperm, and embryo (two plates each, all concentrations are the same).

SEDIMENTATION COEFFICIENTS

COMMON CHARACTERISTICS

The grand average of the sedimentation coefficients for all 40 observations made on isolated β-lectins is about 6 S with most of the individual values between 5 and 7 S. Since the pooled data have this relatively narrow range of population values, the two species have sedimentation coefficient population parameters which are also close to one another. The similarity in certain of these parameters (the population mean, standard deviation, extrema, and range) between the species is visible by inspection of Table II of Appendix II.

INTERSPECIES DIFFERENCES

Despite the similarity of the populations of sedimentation coefficients for each species, differences are discernible between the two. For instance, the Douglas-fir

has a greater minimum (4.9) and maximum (7.9) than the corresponding extrema of loblolly pine (4.6, 6.9); i.e., the ranges overlap but are not identical. Furthermore, although an F-test shows that the variances of the populations are not significantly different from each other, the means follow their ranges' trend, with the Douglas-fir greater than the loblolly pine (6.4 vs. 5.6; Table XXXVII). A Student's t test shows that this difference in the means is significant, in fact, significant even at the 0.1% level of probability. Finally, what is true on the average for the species is also true for the species at each individual developmental state sampled, as Fig. 11 reveals. Table III (in Appendix II) quantifies the varying magnitudes of these species differences between states by listing the ratio (loblolly pine to Douglas-fir, $\overline{L/D}$, always less than unity) and the difference (loblolly pine from Douglas-fir, $\overline{D-L}$, always greater than zero) of the developmental state averages of the sedimentation coefficients. Given the consistency of this mean interspecies difference, it is not surprising that a multiple-regression analysis of variance also confirms its significance.

INTRASPECIES DIFFERENCES

Plant Parts

Inspection of the sapling (SP) data in Table II (Appendix II) does not indicate any striking differences in the sedimentation coefficients of the β-lectins isolated from the plant's three principal parts. Nor are the sapling species' variances significantly different from those of other developmental states characterized, as might be expected if gross differences existed between the various plant part β-lectins. Furthermore, averaging of the individual values (Table XXII, Appendix V) does not cause any distinct pattern to emerge of relative magnitudes of the means. While it is true that the Douglas-fir needles' mean is significantly less than that for the mean of the stems or roots, such is not the case for the loblolly pine or the means

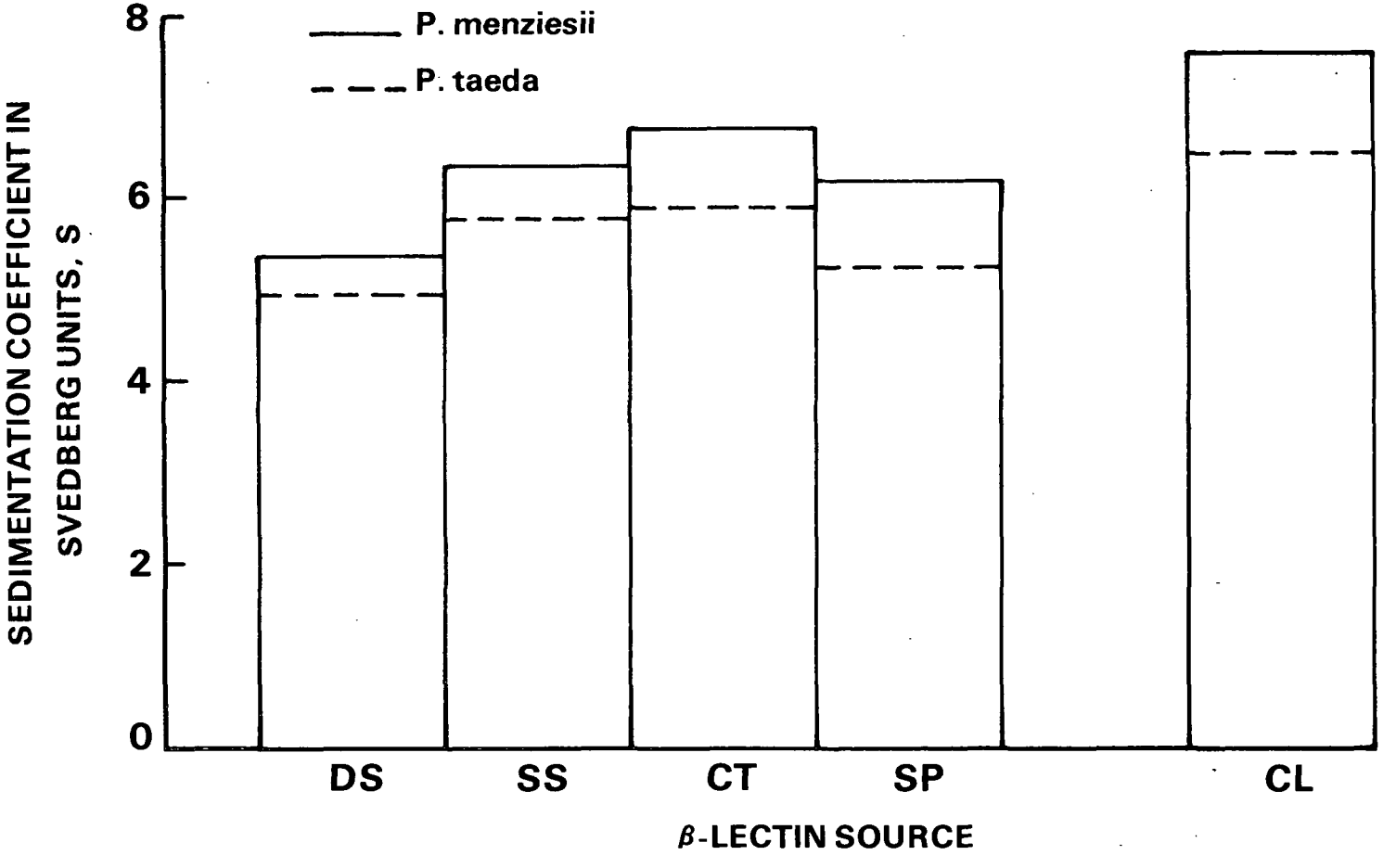


Figure 11. Variation in β-lectin sedimentation on coefficient with development state.

of the pooled data. The overall inference is that no significant differences exist between the β-lectins from these plant parts based on the criterion of mean sedimentation coefficient.

Plant Development

The two-way analysis of variances on the sedimentation coefficient data demonstrates that this parameter varies significantly with the developmental state of the plant from which the β-lectin was isolated. The pattern of this variation is depicted in the histogram of Fig. 11 and graphically in Fig. 12. Note that the ranking of the means by increasing magnitudes for the developmental states are the same for each species and the pooled data (Table III, Appendix II). The trend is for the mean sedimentation coefficient to increase with time from the dry seed through the cotyledon-seedling stages of the plant's life and thereafter to decrease. The callus state has the maximum mean value for each species. A Duncan's multiple range test on the pooled data (Table III, Appendix II) shows the degree of statistical distinctness of these developmental states. The independence of this developmental trend from the species studied is implied by the lack of significance of the interaction term in the analysis of variance. That this trend is a truly developmental one is further indicated by the numerical similarity of the dry seed (DS) normalized data from each species (Table III, Appendix II).

PERCENT PROTEIN

COMMON CHARACTERISTICS

The grand average of the percent protein for all 48 observations made on these Pinaceae β-lectins is about 9% with the individual values ranging from approximately 1 to 30%. The broad range and large standard deviation of the grand average (cf.

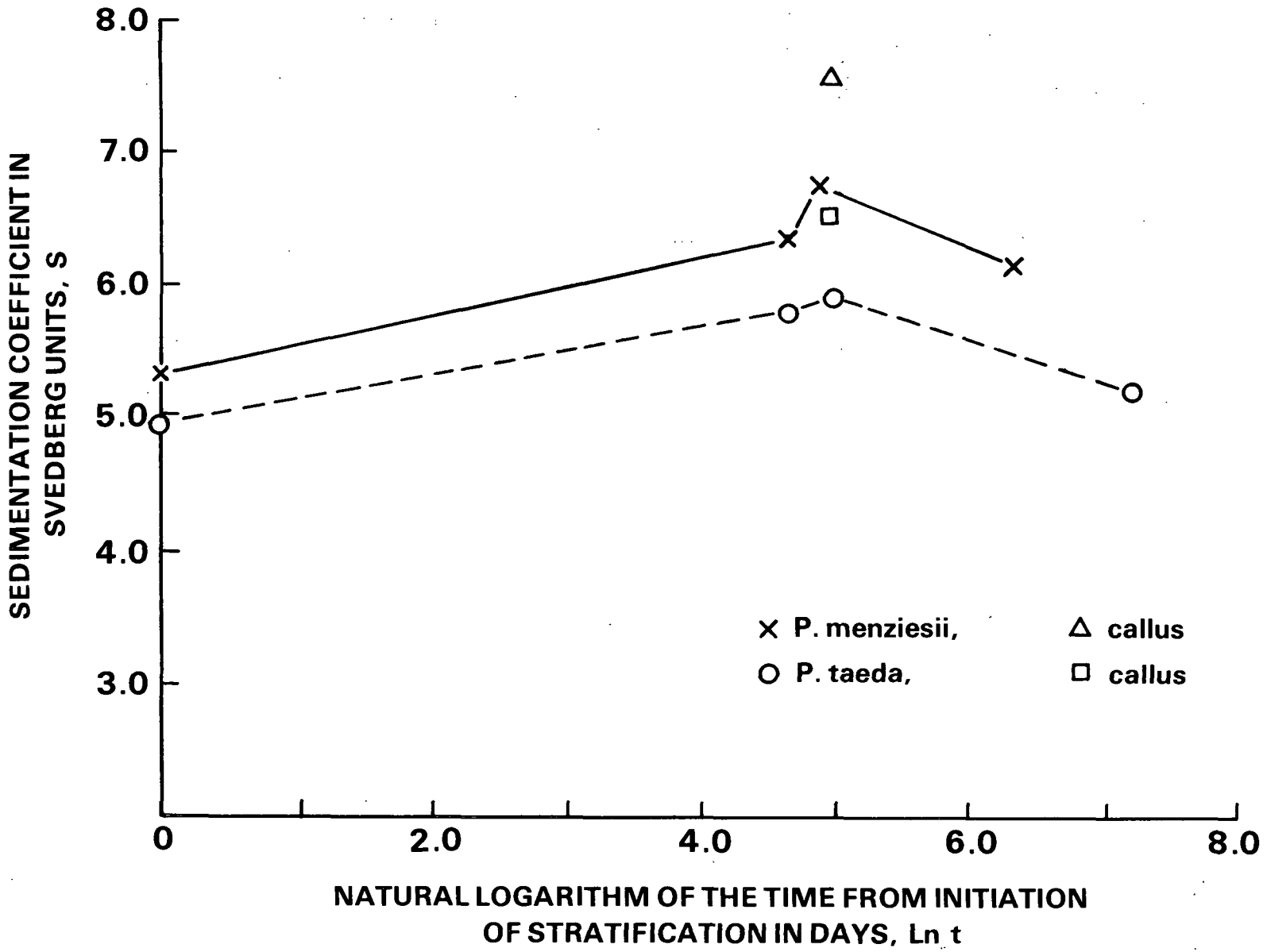


Figure 12. β-lectin sedimentation coefficient vs. ln t (days since stratification initiated; for the callus, days since callus initiated).

Table IV, Appendix III) suggest that the amount of protein in a preparation of β-lectin is strongly dependent on the origin of the sample. Nevertheless, all of the samples did contain protein, and the weight of the protein moiety never exceeded a third of the sample's weight.

INTERSPECIES DIFFERENCES

Unlike the sedimentation coefficient, the principal cause of variation in the pooled percent protein data is due to the species studied. That is not to say that similar trends do not occur, only that the species differences within these trends are more pronounced for the percent protein. For this parameter also the Douglas-fir has a greater minimum (1.6) and maximum (30.2) than the corresponding extrema of loblolly pine (0.7, 19.0), but the overlap of the ranges is less than that of the sedimentation coefficient. The standard deviation of the Douglas-fir is again greater than that of the loblolly pine, so much so that this time the variances are significantly different. Despite the inequality of the variances, a modified Student's t-test shows that the species means are significantly different. And, once again, what is true on the average for the two species is also true for them at each individual state sampled (Fig. 13). Table V (in Appendix III) quantifies the varying magnitudes of these species differences between states by listing the ratio (loblolly pine to Douglas-fir, $\overline{L}/\overline{D}$, always less than unity) and the difference (loblolly pine from Douglas-fir, $\overline{D}-\overline{L}$, always greater than zero) of the developmental state averages of the protein percentages. Here, too, relative differences are greater and ratios are less than for the sedimentation coefficient. In view of all the foregoing evidence, it is not surprising that a multiple-regression two-way analysis of variance confirms that species is a main effect on the protein content of the Pinaceae β-lectins (Table XXXVII, Appendix VIII).

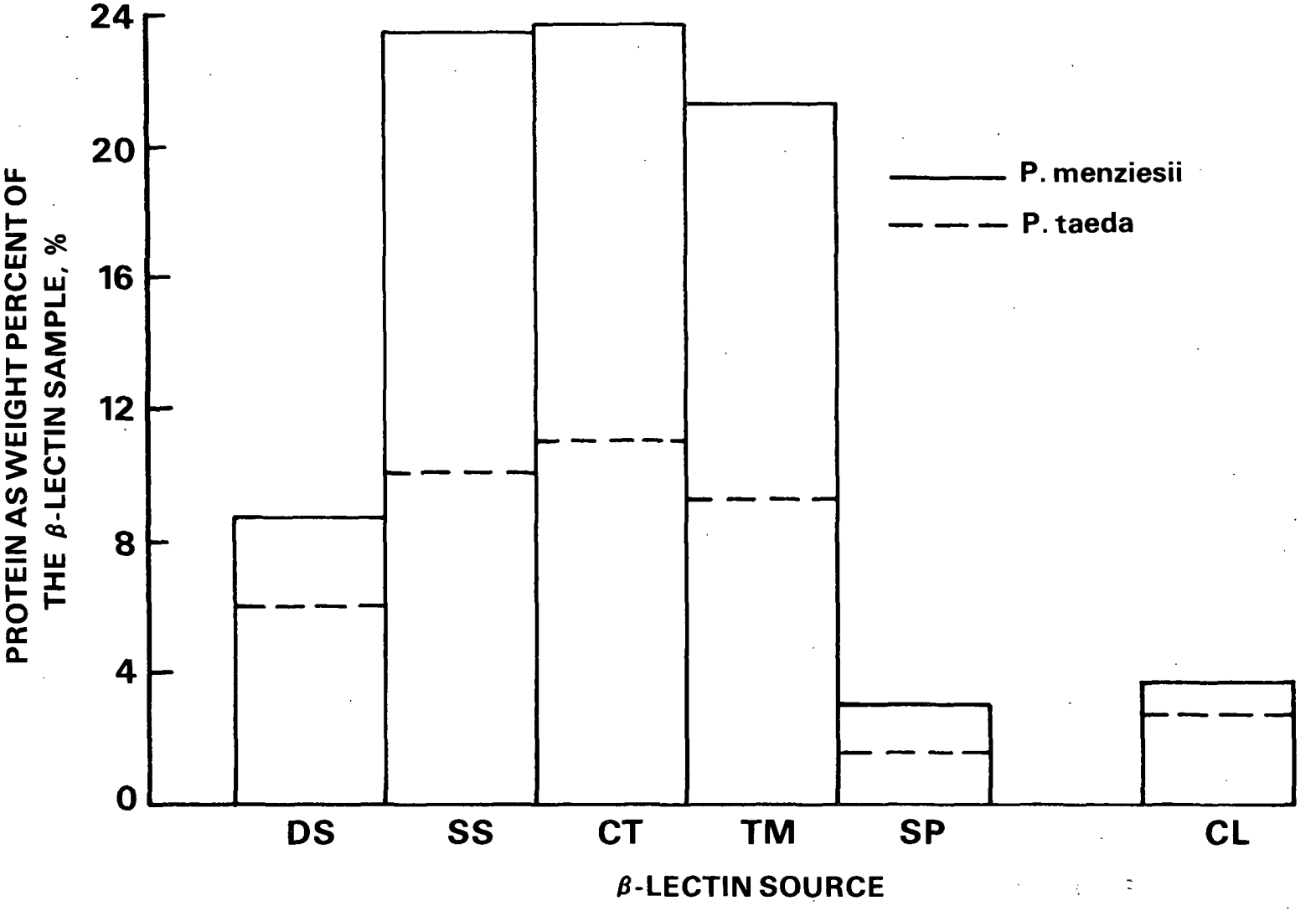


Figure 13. Variation in β-lectin weight percent protein with developmental state.

INTRASPECIES DIFFERENCES

Plant Parts

Inspection of the sapling (SP) data in Table IV (Appendix III) does not alert one to any major differences in the weight percent protein from the plant's three principal parts. Nor are the sapling species' variances significantly different from those of other developmental states characterized, as might be expected if extreme differences existed between the various plant part β-lectins. Moreover, averaging of the individual values (Table XXII, Appendix V) does not serve to accentuate any distinct order of magnitudes for the means. None of the three *t* tests constructable on the data (Table XXII, Appendix V) showed a significant difference in the means for either species or the pooled data. Even when the corresponding values from the two months of data are averaged with the sapling data, the means of the percentage protein of the plant parts' β-lectins still are not significantly different one from another. Overall, then, no significant differences appear to exist between the β-lectins of needles, stems, and roots based on the criterion of mean percent protein.

Plant Development

The two-way analysis of variance on the percent protein data demonstrates that this parameter varies significantly with the developmental state of the plant from which the β-lectin was isolated. The pattern of the variation is depicted in the histogram of Fig. 13 and graphically in Fig. 14. Table V (Appendix III) displays data used to construct these plots; note that as with the mean sedimentation coefficients for the developmental states, the ranking of the means by increasing magnitudes is the same for each species and the pooled data. Likewise similar to the sedimentation coefficient, the trend for the mean percent protein is to increase with time from the dry seed through the cotyledon-seedling stages of the plant's life and then to decrease. Two striking differences do exist, however, between the trend behavior of these parameters. The first is that the magnitude of the rise and fall

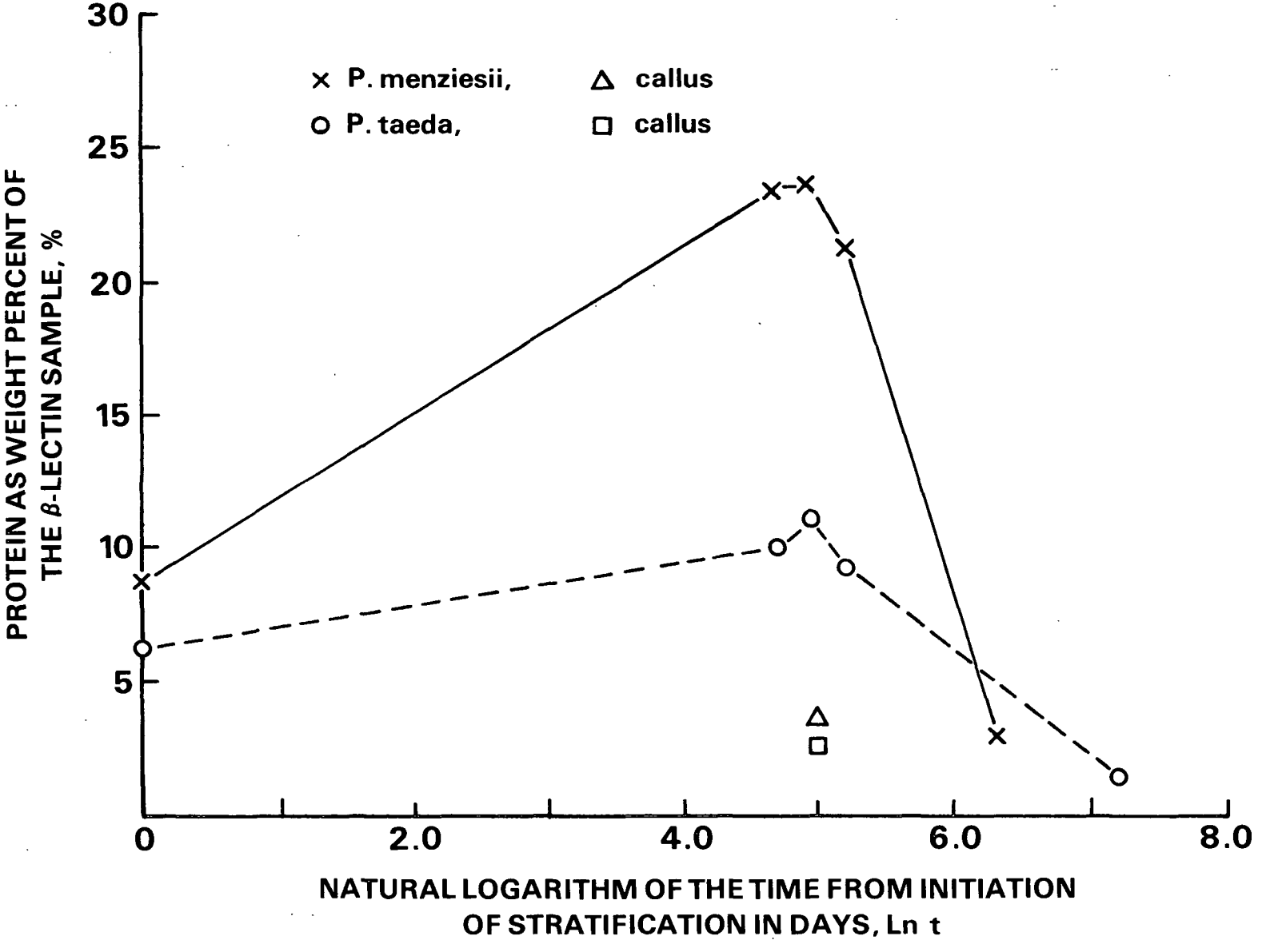


Figure 14. β-lectin weight percent protein vs. ln t (days since stratification initiated; for the callus only, days since callus initiated).

of the parameter with the plant's development is greater for the mean percent protein than the mean sedimentation coefficient. Second, the callus state represents the penultimate minimum for the percent protein parameter, whereas it is the maximum for the sedimentation coefficient parameter. A Duncan's multiple range test on the pooled data (Table V, Appendix III) shows the number of distinctly separate developmental states to be fewer than those for the sedimentation coefficient and with less overlap. The lack of overlap here can be ascribed to the abruptness of the transitions from the dry seed to the stratified seed and from the two-month seedling to the sapling or to the callus states. Although changes are abrupt for both species, they are greater in Douglas-fir than loblolly pine as is apparent by comparing the dry seed normalized data (Table V, Appendix III). That is, the two species are convergent with respect to the mean protein percentage of the β-lectins (have numerically close values for the normalized means) for the sapling (SP) and callus (CL) states, but divergent with respect to the stratified seed (SS), cotyledon-seedling (CT), and two-month seedling (TM) states. It is this convergence of developmental trend in the two species which accounts for the significance of development as a main effect, just as it is this divergence which accounts for the significance of the interaction term in the analysis of variance (Table XXXVII, Appendix VIII).

AMINO ACID ANALYSES

COMMON CHARACTERISTICS

The protein moiety of the Douglas-fir and loblolly pine β-lectins was found to consist of 19 amino acid residues, with the same relative abundance of each residue being preserved from sample to sample (Tables VI-XVIII, Appendix IV). On the basis of the total average (Table XIX, Appendix IV), five of these residues (glutamate, glycine, serine, aspartate, and alanine) comprise 55% of the protein. Each of these five individually is greater than 10% (mole fraction) of the total protein. Another

six residues (leucine, arginine, lysine, valine, threonine, and isoleucine) comprise 33% of the total protein and individually are greater than 4% of the total protein. Finally, the remaining eight residues (phenylalanine, tyrosine, proline, hydroxyproline, methionine, ornithine, histidine and half cystine) comprise only 12% (individually less than 3%) of the total protein.

A one-way analysis of variance was run on the 19 residues over the total data (the 48 determinations on separate preparations of β-lectins). The means of the residues were found to be significantly different from one another, significant at well below even the 0.1% level of probability. A Duncan's multiple range test on the data shows that there is a relatively small amount of overlap between residue means (Table XIX, Appendix IV). There are 14 separate groupings of the 19 different means, seven of these being unique (seven different residue means in groups by themselves). Not surprisingly, most of the overlap that does occur is in the minor residues. Figure 15 emphasizes the similarity of the two species' mean β-lectin amino acid compositions.

INTERSPECIES DIFFERENCES

The 19 two-way analyses of variance on the amino acid residues revealed virtually no significant differences in amino acid composition of the β-lectins related to their species of origin. Of the 19 residues, only two (hydroxyproline and aspartate) are significantly different. For Douglas-fir, the mean hydroxyproline content is greater than that for loblolly pine (2.2 vs. 0.8 mole percent). The hydroxyproline difference seems to be principally related to the callus state, where the highest difference in the species means occurs for any of the residues (7.9% vs. 1.3%). However, the mean hydroxyproline content of the Douglas-fir is never less than that of the loblolly pine for any of the six developmental states sampled, being greater in four of them and equal in two (Table XVIII, Appendix IV). In contrast to this, the mean aspartate content of the loblolly pine was never so dramatically different

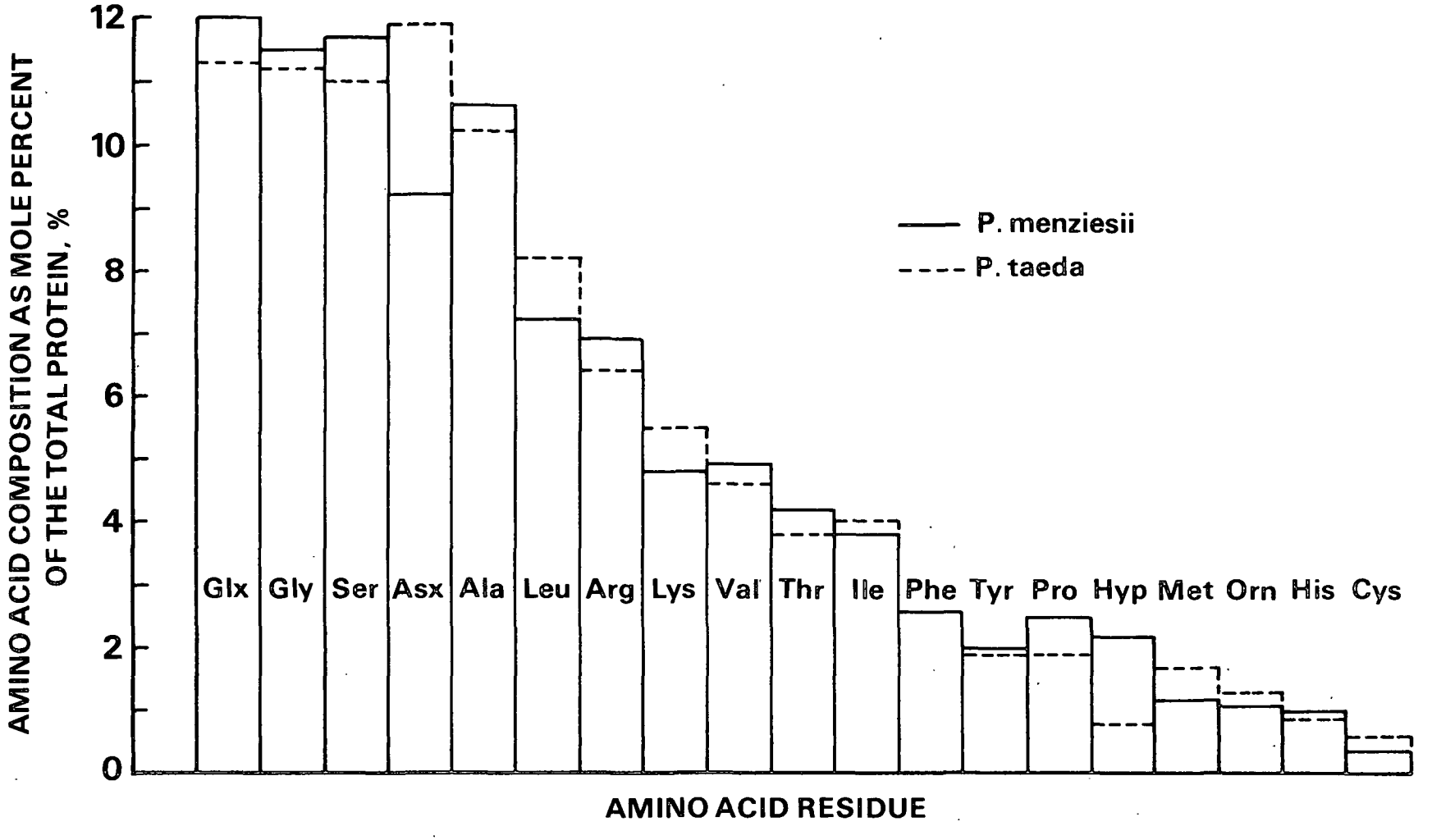


Figure 15. Interspecies comparison of amino acid analyses.

than that of Douglas-fir for any one developmental state [species difference: 9.2% (D) vs. 11.9% (L)]. On the other hand, the interspecies differences were on the average larger (2.5% for the aspartate vs. 1.4% for the hydroxyproline) and more consistent (with the Douglas-fir mean aspartate content greater than the loblolly pine mean aspartate content) across the developmental states (loblolly pine mean being greater than the Douglas-fir mean in five of the six states sampled).

INTRASPECIES DIFFERENCES

Plant Parts

The amino acid compositions from the individual analyses of the β-lectins of needles, stems, and roots (two-month seedling and sapling material: Tables IX, X, XV, and XVI) do not appear very different from one another. Nor are any extreme differences apparent in the respective means of the plant part samples (Table XXI, Appendix V). Furthermore, when hydroxyproline and aspartate (the two residues which were shown to be significantly different between species and perhaps generally sensitive indicators of differences in β-lectin amino acid composition) were tested for differences in the plant parts, none were detected (Table XXII, Appendix V). Overall, then, the amino acid compositions from the three plant parts seem to be equivalent.

Plant Development

By two-way analyses of variance, 16 residues were found to have means which differed significantly with developmental state (the three exceptions were leucine, isoleucine, and methionine). However, there does not seem to be any developmental trend in these state changes; a residue may first increase, then decrease, then increase again, etc., with successive developmental states. Also, for about half the residues showing significant differences with developmental state, the differences are not statistically great, being spread over only two groups with overlap. Thus, it seems likely that if a larger number of equal observations were made on the states

(only 6 to 12 observations were made for the means of Table XIX), the state dependent differences would prove as insignificant as those between the two species (where just two groups were compared; the loblolly pine means were based on 26 observations vs. the Douglas-fir means based on 22 observations: cf. the means and standard deviations of Table XVIII, Appendix IV, and Fig. 15).

CARBOHYDRATE ANALYSES

COMMON CHARACTERISTICS

The grand average (of all 34 determinations reported in Tables XXIII-XXXIV, Appendix VI) for the neutral sugar compositions provided a general idea of the character of the carbohydrate moiety of these Pinaceae β-lectins. Thus inspection of Table XXXIII, Appendix VI, confirmed the reported arabinogalactan nature of the β-lectins with the sum of galactose to arabinose comprising 80% of the neutral sugars and with a galactose to arabinose ratio of 1.9. Although eight sugars were found in both species at approximately the same mole percentage per component, they were not equally abundant. The eight sugars found in decreasing order of abundance were galactose, arabinose, glucose, rhamnose, mannose, xylose, ribose, and fucose (Fig. 16). Galactose, arabinose, and glucose were the major sugars found and together constitute 93% of the total neutral sugars. The other five components were always minor sugars. Of these, rhamnose, mannose, and xylose were generally present. The grand average calculation showed rhamnose present at twice the level of mannose (4% vs. 2%), with xylose less than these (ca. 1%). Ribose and fucose were sometimes found in trace amounts.

INTERSPECIES DIFFERENCES

Two-way analyses of variance on the neutral sugars (mole percent basis) on the whole did not reveal any striking differences in the two species with respect to

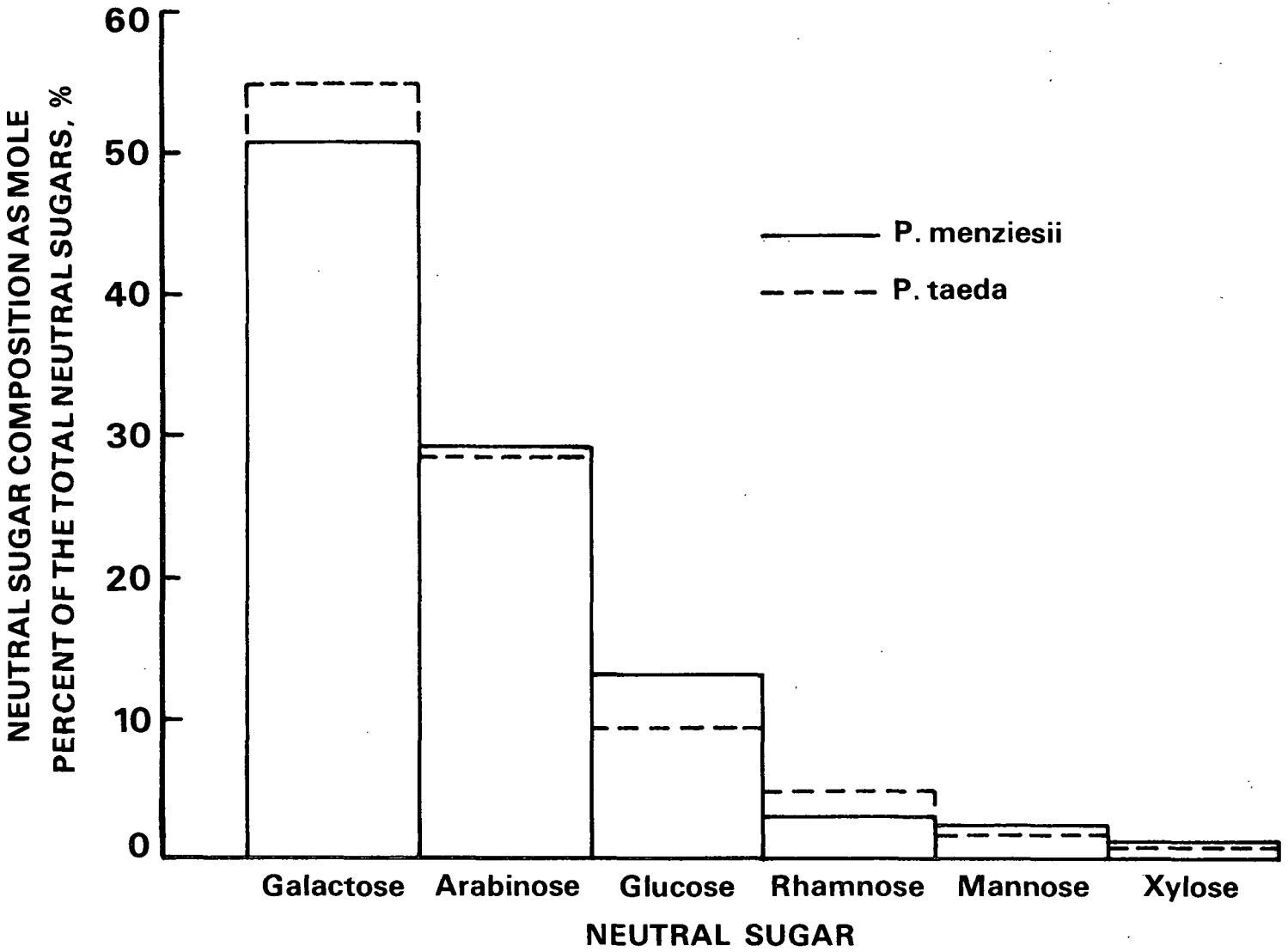


Figure 16. Interspecies comparison of neutral sugar composition (mole percent basis).

these parameters. Two exceptions to this similitude in the species neutral sugar analyses were for the mole percent galactose and the total neutral sugars as weight percent of the sample. Loblolly pine was more abundant in the levels of each of these parameters than Douglas-fir. For the mole percent galactose, this species difference was evident in all of the developmental states sampled except for the dry seed state (where the two were approximately equal), as well as in the species average (Table XXXIII, Appendix VI, and Fig. 16). For the total analyzed carbohydrate, the mean of the Douglas-fir to loblolly pine values was 77.7%. The magnitude of this difference and its statistical significance warranted putting the individual determinations on an absolute basis. When this was done and the overall means calculated (Table XXXV, Appendix VI) it could be seen that the neutral sugars in loblolly pine were always greater than or equal to that of Douglas-fir. Furthermore, analyses of variance of the data on the absolute basis showed that arabinose as well as galactose was present in smaller amounts in the Douglas-fir β-lectins than in those from loblolly pine (Fig. 17). All of these considerations indicated that the β-lectins of loblolly pine have more extensive carbohydrate moieties (particularly with respect to galactose) than those of Douglas-fir.

INTRASPECIES DIFFERENCES

Plant Parts

Tables XXVI and XXXI (Appendix VI) revealed no enormous differences in the neutral sugar composition of the β-lectins isolated from needles, stems, and roots of the saplings for either species. Although the β-lectins from Douglas-fir roots seemed to have a lesser galactose and greater glucose content than that of the β-lectins from the needles or stems, *t* tests showed that these differences were not significant. The individual determinations of the loblolly pine plant part β-lectins did not even suggest any differences between their respective carbohydrate moieties, nor did the results of significance tests on the means contradict this

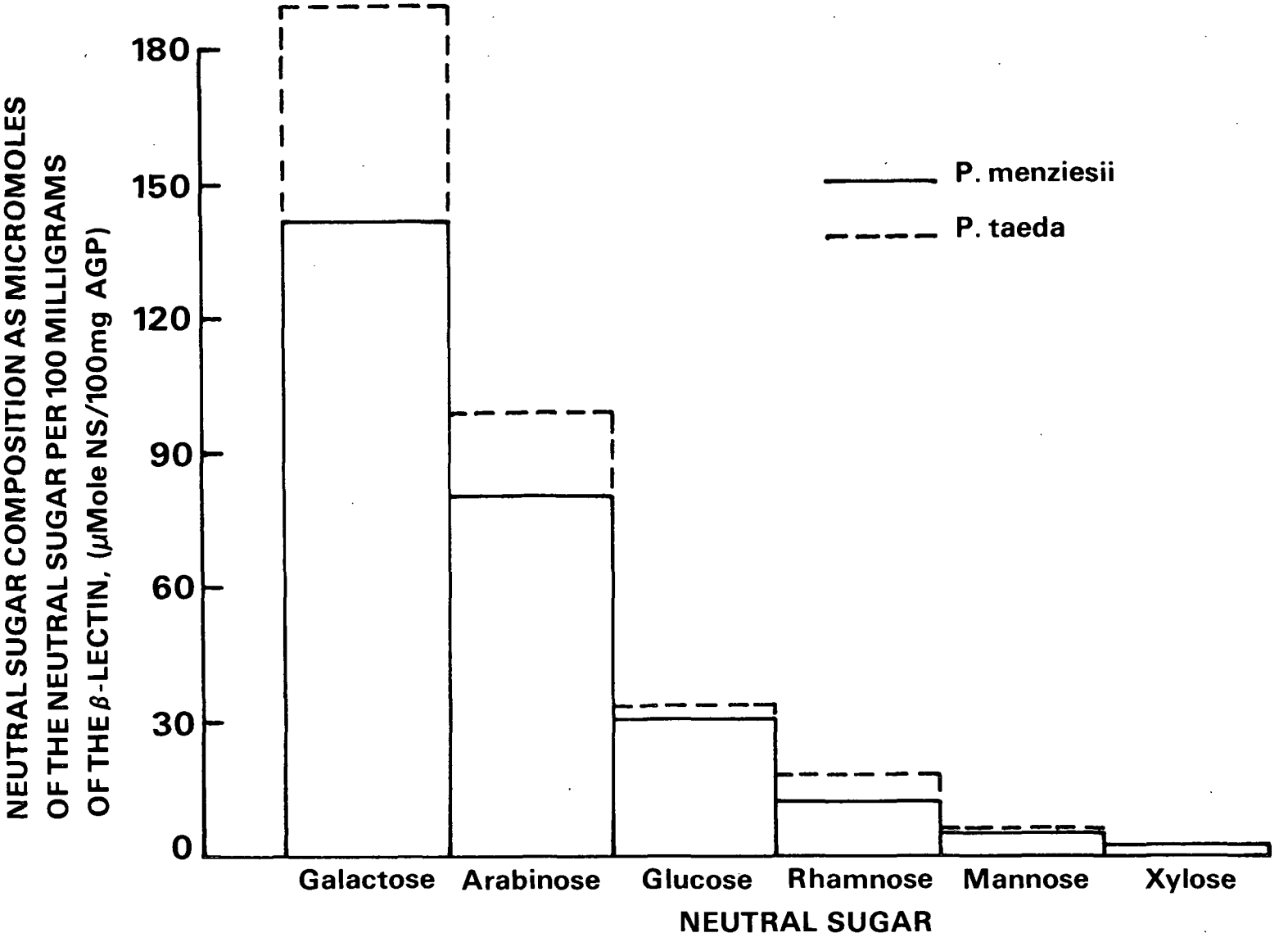


Figure 17. Interspecies comparison of neutral sugar composition (absolute basis).

first impression (Table XXII, Appendix V). Finally, the ratio of nongalactose sugars to galactose was also not significantly different among these β-lectins. In short, the evidence presented here did not indicate any differences in the β-lectins of plant parts based on their respective neutral sugar compositions.

Plant Development

Two-way analyses of variance showed significant differences in the means of the neutral sugars with developmental state (Table XXXVII, Appendix VIII). Furthermore, there was a pattern in the changing of the means which was followed by both species. Figure 18 demonstrates the nature of these changes, a gradual decrease in the relative galactose content of each species' β-lectins, with a concomitant increase in arabinose and glucose. Also statistically significant were the increasing values with time of the ratios of arabinose to galactose and total nongalactose sugars to galactose for both species, Fig. 19. When the species' data were pooled (all of the individual observations averaged together), the trend could be summarized in either of the two ways illustrated in Fig. 20.

ISOELECTRIC FOCUSING

COMMON CHARACTERISTICS

The electrofocused Pinaceae β-lectins stained unequally with three different stains. The β-lectins were most sensitive to the specific Yariv glucoside (β-GLU), less so to the carbohydrate stain (PAS), and least to the protein stain (Coomassie Brilliant Blue). Figure 21 illustrates the type of results obtained with these stains. In addition to confirming the glycoprotein nature of the β-lectins, the stains also revealed that there was a multiplicity of components in each β-lectin.

It cannot be said at present exactly how many distinct components actually are present, since electrofocusing is not yet completely understood. Thus,

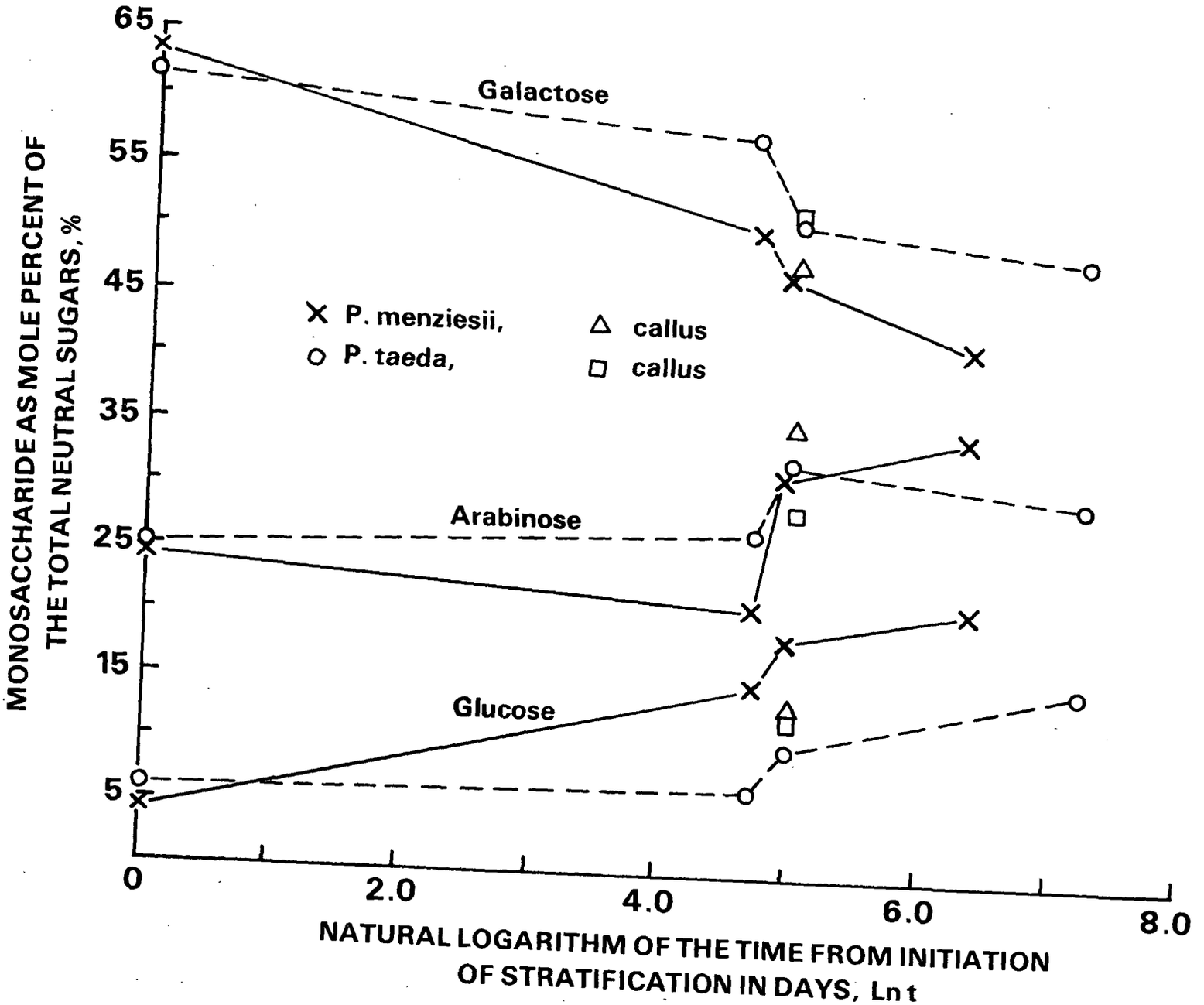


Figure 18. Interspecies comparison of changing sugar composition with time (mole percent basis), for the callus, t is the time from its initiation.

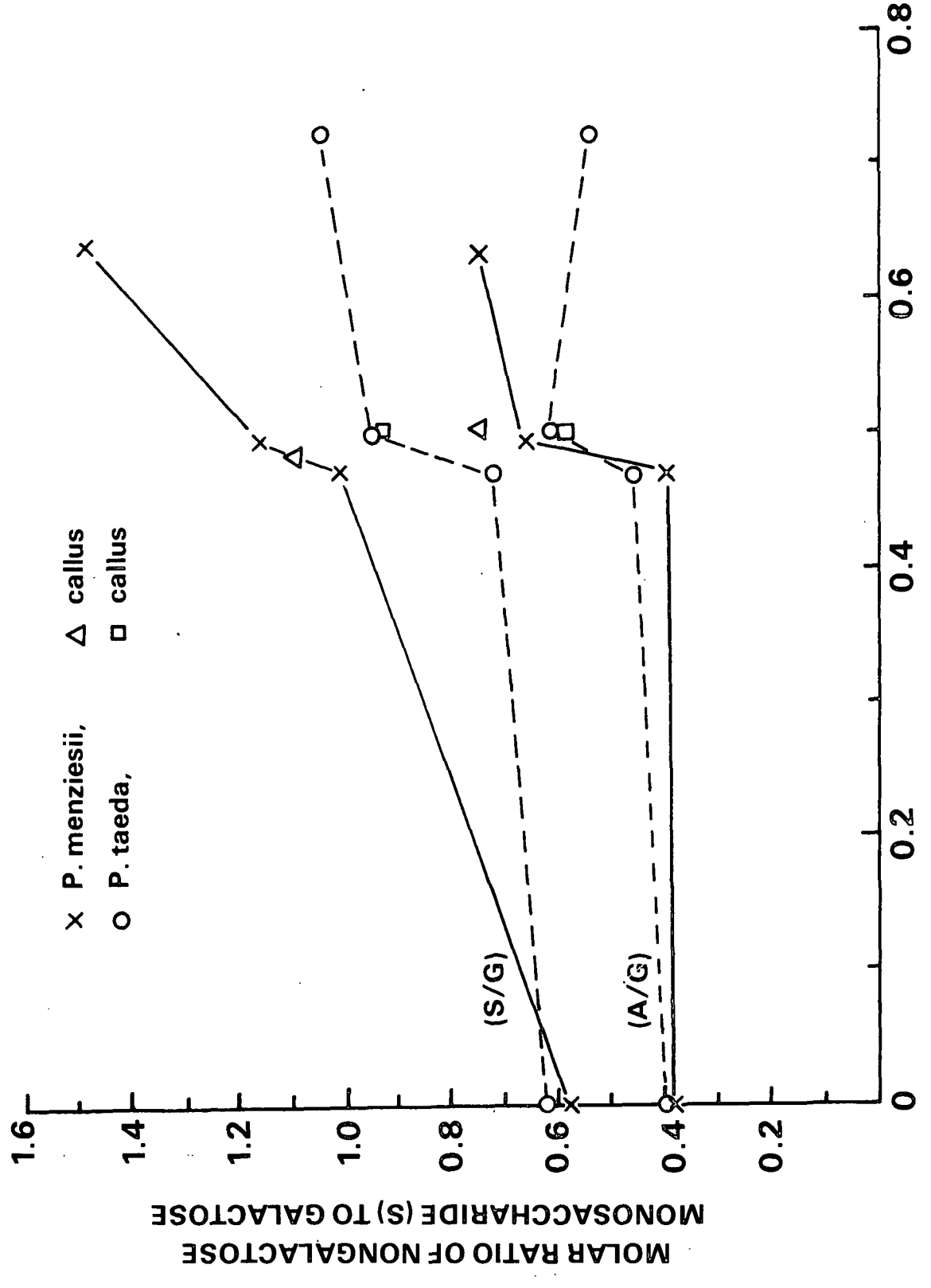


Figure 19. Interspecies comparison of changing sugar composition with time (mole ratio basis), callus is plotted vs. ln t where t is the time from its initiation to harvest.

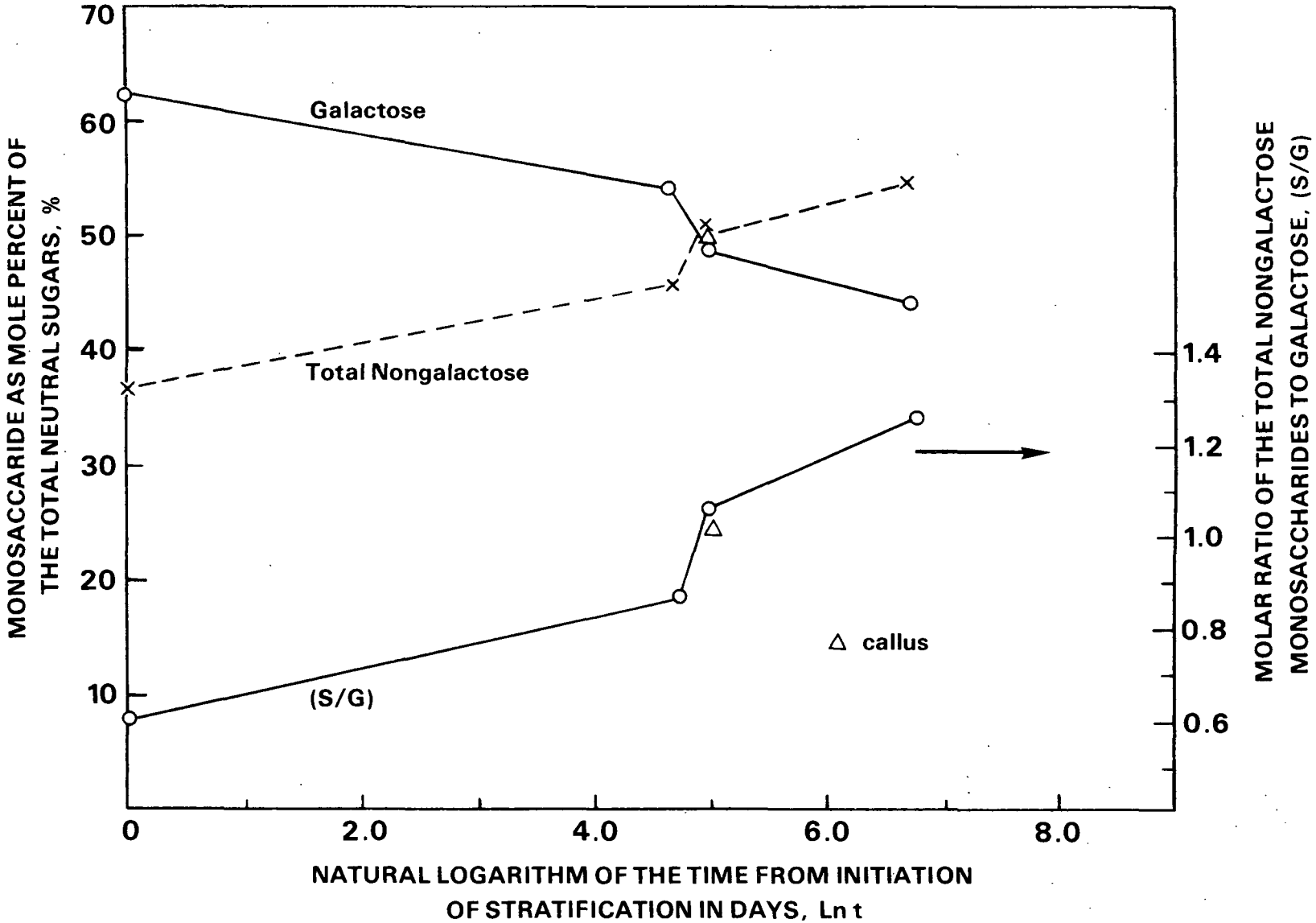
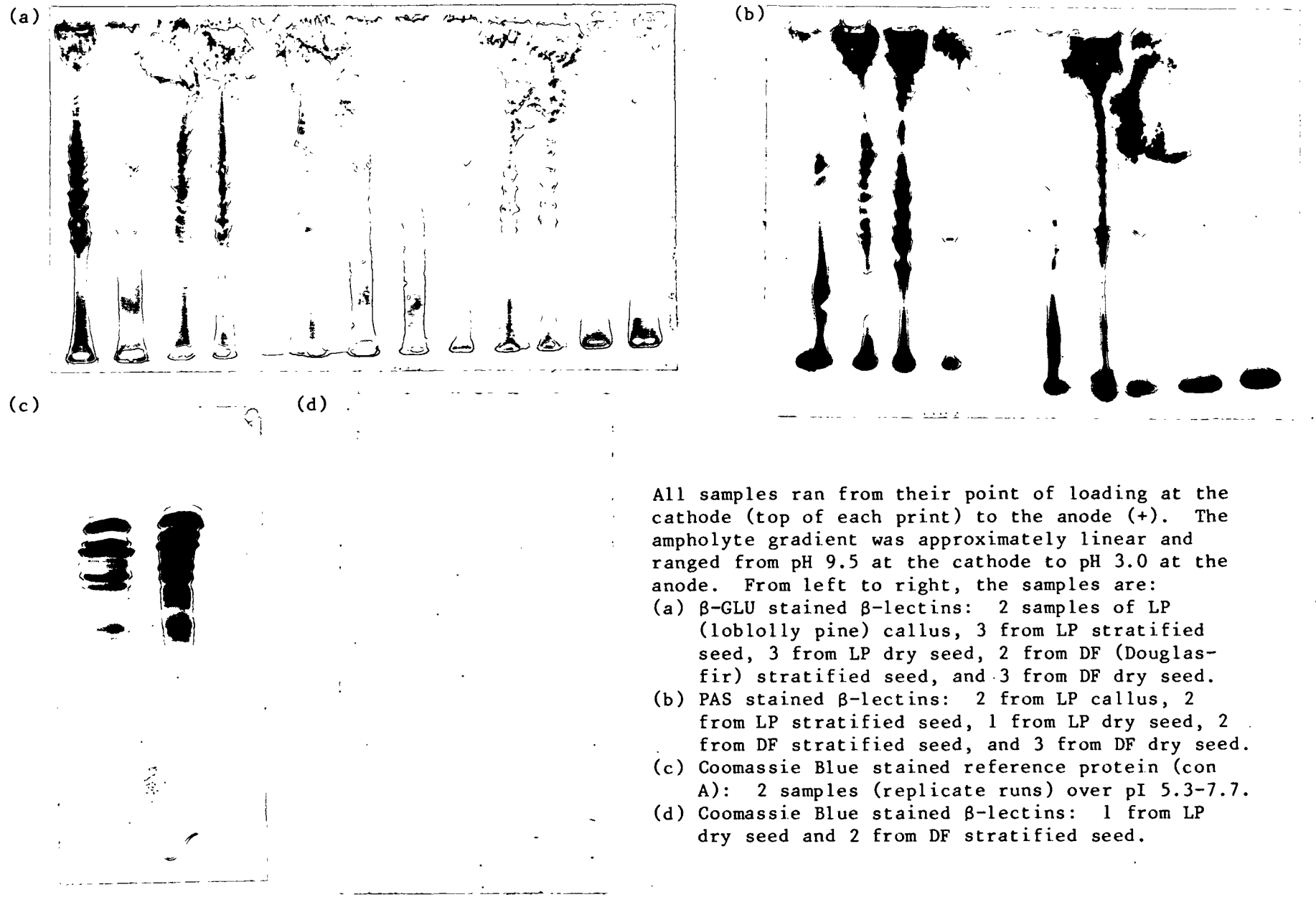


Figure 20. Overall carbohydrate trend with time (for callus, time since its initiation).



All samples ran from their point of loading at the cathode (top of each print) to the anode (+). The ampholyte gradient was approximately linear and ranged from pH 9.5 at the cathode to pH 3.0 at the anode. From left to right, the samples are:

(a) β-GLU stained β-lectins: 2 samples of LP (loblolly pine) callus, 3 from LP stratified seed, 3 from LP dry seed, 2 from DF (Douglas-fir) stratified seed, and 3 from DF dry seed.

(b) PAS stained β-lectins: 2 from LP callus, 2 from LP stratified seed, 1 from LP dry seed, 2 from DF stratified seed, and 3 from DF dry seed.

(c) Coomassie Blue stained reference protein (con A): 2 samples (replicate runs) over pI 5.3-7.7.

(d) Coomassie Blue stained β-lectins: 1 from LP dry seed and 2 from DF stratified seed.

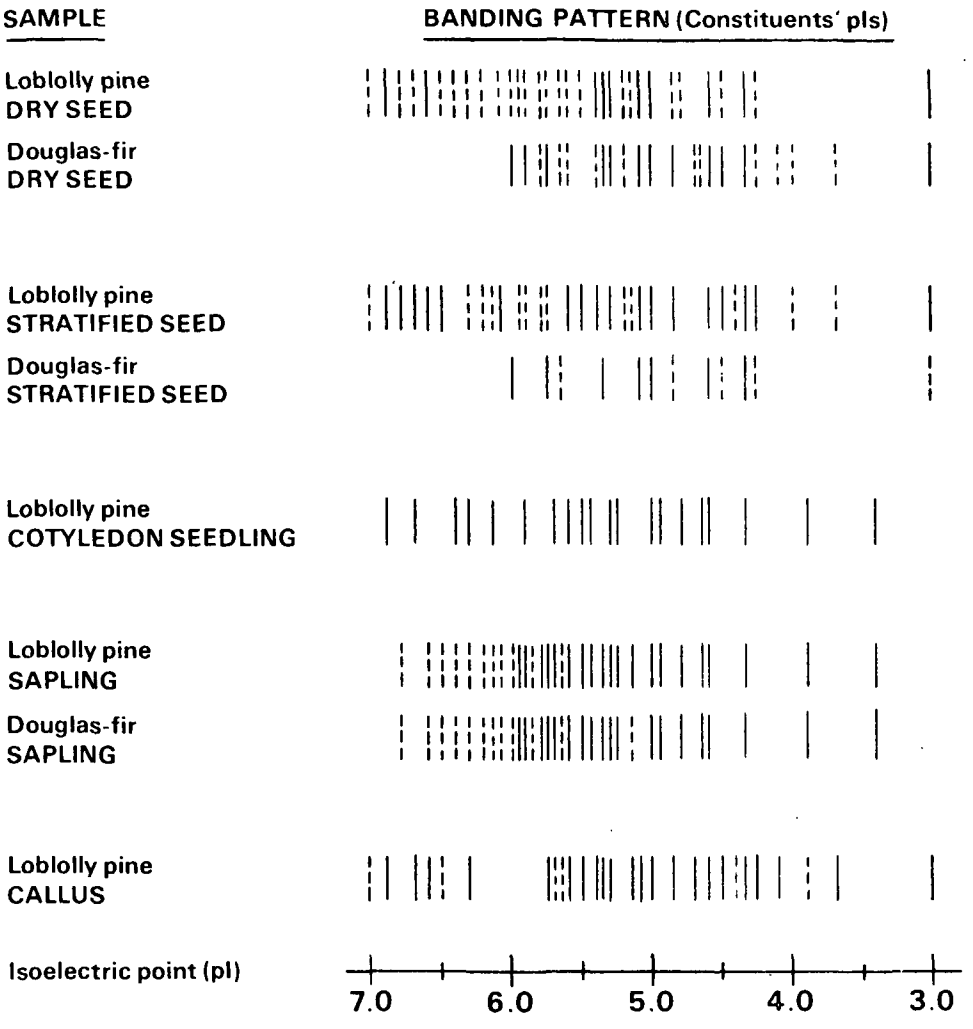
Figure 21. Electrofocused β-lectins and reference protein (con A).

concanavalin A, an ostensibly well-characterized protein of four tetrameric strands gave 47 bands when electrofocused as a standard (Fig. 21c). Despite such problems of physical interpretation, electrofocusing is useful for characterizing and identifying complex molecular assemblies. To this end the Yariv glucoside was employed, since it stained more of the electrofocused material more clearly than either of the nonspecific stains.

Table XXXVI (Appendix VII) lists the banding patterns (exclusive of the frequently appearing multitude of faint bands in the region of pI 7.0-8.0) representing β-lectins from eight different sources. Always visible were 13-18 strong bands, with as many more weak bands and heavy staining at the anode. The prominent bands were usually in the region pI 4.3-6.0, the region where the banding tended to be the same from sample to sample regardless of species or developmental state. In particular, bands were always present at pI 4.35, 4.60, and 5.00 and virtually always present at pI 4.80, 5.30, 5.60, and 5.75. The pattern in this region (cf. Fig. 22) seems to be diagnostic of a Pinaceae β-lectin and is possibly common to all β-lectins. In contrast to this common banding region, the regions of pI 2.3-4.3 and pI 6.0-8.0 may be "fingerprint" regions whereby β-lectins from a given source can be distinguished from one another.

INTERSPECIES DIFFERENCES

No outstanding differences were apparent from the data presented here. Possibly for the dry seed and stratified seed β-lectins, loblolly pine has more bands in the pI 6.0-7.0 region than Douglas-fir. Certainly for the sapling β-lectins the banding pattern for the two species was the same, even in the faint higher pI region. If more interspecies developmental state comparisons could be made, it might well turn out that identity of pattern would be established for these also. Unfortunately, due to a lack of data, this question cannot be unequivocally decided at present.



ISOELECTRIC FOCUSING OF PINACEAE β-LECTINS

The eight banding patterns shown above have been abstracted from two to three determinations (using separate preparations of β-lectins) on a given source. Concentrations used ranged from 5 to 15 mg/mL. A 1 mg/mL solution of β-GLU in 1%(w/v) NaCl was used to stain the gels after electrofocusing. Destaining was accomplished by washing in distilled water over a few days.

(solid line) indicates that the band was noticeably present in two or more of the samples.

(dashed line) indicates that the band was present in only one of the gels or that it was uniformly weak.

Figure 22. Pinaceae β-lectin banding patterns.

INTRASPECIES DIFFERENCES

Plant Parts

The sapling β-lectins of both species yielded identical banding patterns, regardless of the plant part from which the β-lectin had been isolated.

Plant Development

Differences in the banding patterns of the β-lectins from different developmental states could be picked out from Fig. 22. However, just as with the interspecies comparisons, insufficient data are now available to unequivocally decide whether such differences are genuinely developmental markers or merely due to variations in the electrofocusing trials.

FOURIER TRANSFORM INFRARED SPECTROSCOPY

COMMON CHARACTERISTICS

All the Fourier transform infrared spectra taken on the Pinaceae β-lectins were quite similar to one another. That is, absorptions occurred at similar frequencies in similar fashions (the slope of the absorption trace altered little) from sample to sample of the β-lectins. It was more the variation in the relative intensities of the absorbing bands than gross alterations in their form which made the spectra useful in distinguishing the β-lectin samples from each other.

An assignment of functional groups to the dominant absorptions could be made on the basis of comparison to model compounds and reported information concerning the structure of the β-lectins (32). Thus, comparison of the spectrum of the β-lectin isolated from Douglas-fir stratified seed with that of the protein concanavalin A clearly indicated the presence of the amide I (C=O stretching, 1660 cm^{-1}) and amide II (NH bending, 1540 cm^{-1}) bands from the peptide bond (Fig. 23). Likewise, the carbonyl stretching peak of glucuronic and galacturonic acids lay approximately at the

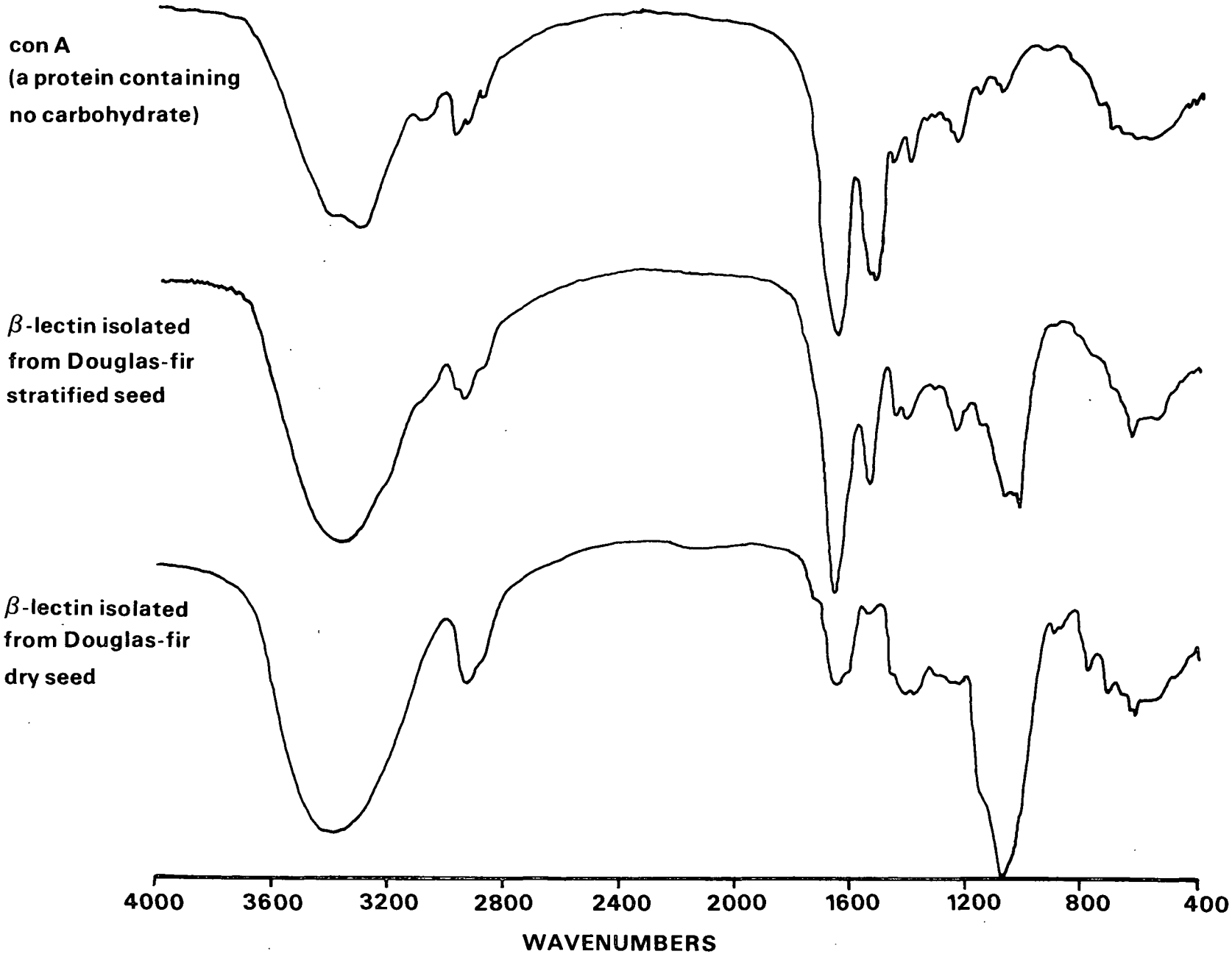


Figure 23. Fourier transform infrared transmittance spectra of amide peaks (ca. 1660 and 1520 cm^{-1}).

same location (1730 cm^{-1}) as the minor peak to the left (shorter wavelength) of the amide carbonyl (Fig. 24). The existence of this 1730 cm^{-1} band probably indicates the presence of uronic acids, as has been reported in Phaseolus vulgaris and Nicotiana β-lectins (32,33). Macromolecular precedent for such a double carbonyl assignment to the two absorptions noted in this region occurs in the literature, e.g., in the spectrum of poly-γ-benzyl-L-glutamate the absorption at 1659 cm^{-1} is assigned to the amide carbonyl and that at 1735 cm^{-1} to the ester carbonyl (74). In a similar way, the ubiquitous broad absorption around 3400 cm^{-1} is undoubtedly due to intermolecularly hydrogen-bonded OH and NH stretching with other absorptions around 1420 cm^{-1} (scissoring), 1250 cm^{-1} (wagging and twisting), and 600 cm^{-1} (rocking). Occasionally being resolvable as a peak, the shoulder at 1140 cm^{-1} is the characteristic absorption for cyclic ethers (CO) of six-membered rings (75). For diagnostic purposes with these β-lectins, however, the second most useful absorption (after the amide I band) is the CO stretching of the alcohol groups. The primary alcohol group (RCH_2OH) absorbs at 1030 cm^{-1}, whereas the secondary alcohol group (R_2CHOH) absorbs at 1080 cm^{-1} (75).

INTERSPECIES DIFFERENCES

No consistent marker common to all the infrared spectra of Douglas-fir alone nor one commmon to all the spectra of loblolly pine alone could be found. Nevertheless, some spectral differences were apparent when corresponding developmental states of each species were compared. At this time, limited data only allows apparently valid interspecies comparison between two developmental states. The less diagnostically useful of these two states is the sapling due to the extreme similarity of the six spectra (Fig. 25 and 26). In fact, the spectrum from the Douglas-fir sapling needle β-lectin is virtually superimposable on that of the loblolly pine sapling root β-lectin. However, two of the Douglas-fir samples do differ in two respects from the loblolly pine sapling β-lectins. First, the Douglas-fir spectra have distinct shoulders at 1140 cm^{-1}, whereas only a gradual broadening of the COH stretching

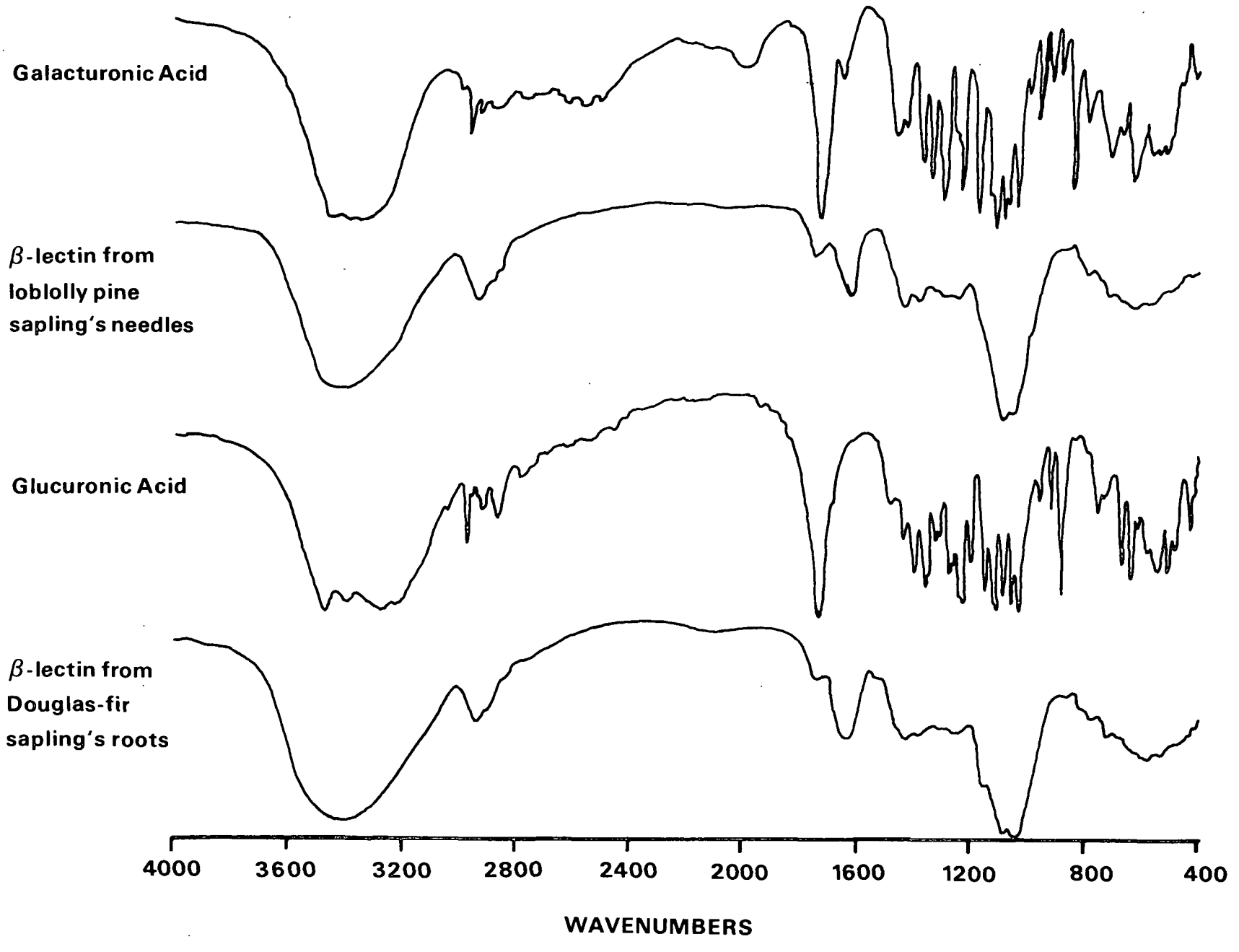


Figure 24. Fourier transform infrared transmittance spectra of acid carbonyl peaks (ca. 1730 cm^{-1}).

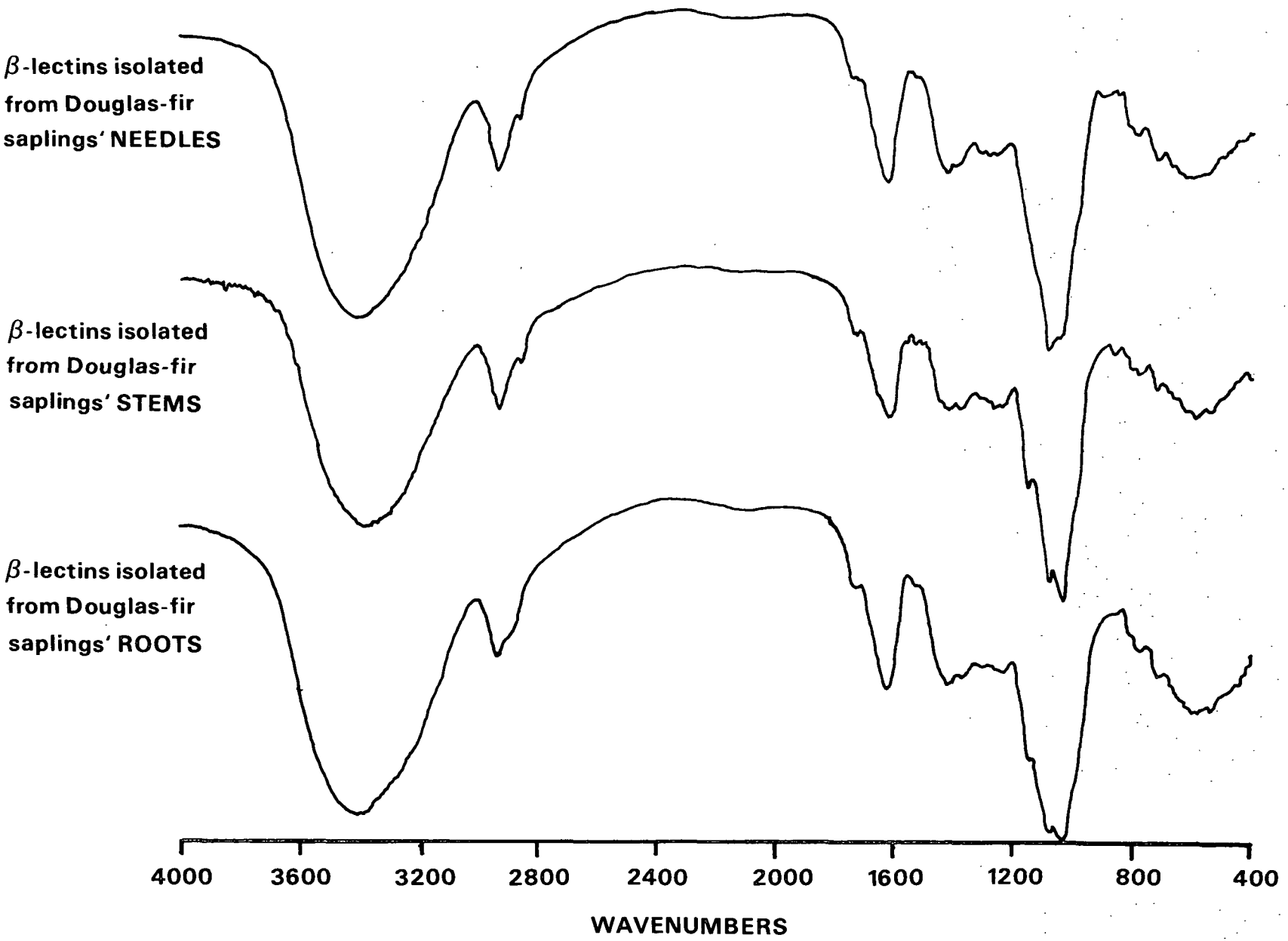


Figure 25. Intraspecies comparison: Douglas-fir (FTIR transmittance spectra of β-lectins from plant parts).

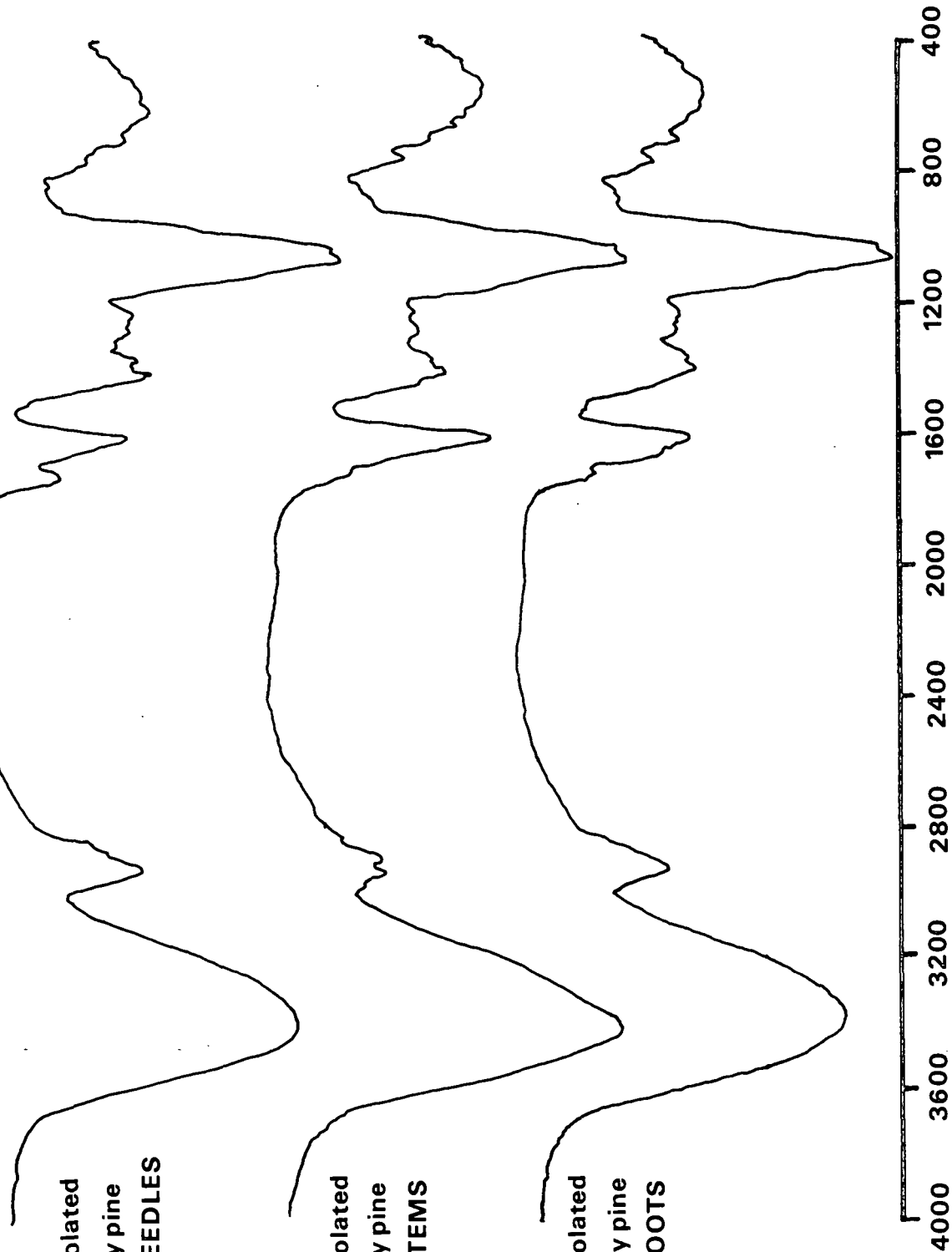


Figure 26. Intraspecies comparison: loblolly pine (FTIR transmittance spectra of β-lectins from plant parts).

absorption is noted in the loblolly pine spectra. Second, the absorption for primary alcohol stretching (1030 cm^{-1}) in these same two spectra is more intense than that of the absorption for secondary alcohol stretching (1080 cm^{-1}), an occurrence which was not observed for any of the loblolly pine spectra taken. Repetition of the experiment (spectra were taken on the β-lectins from a separate extraction of Douglas-fir sapling needles, stems, and roots) again revealed the relative intensification of the 1140 cm^{-1} and 1030 cm^{-1} peaks. If the spectral species distinctness of the β-lectins from the saplings is equivocal, such is not the case for the β-lectins from the stratified seeds of the two species. Even a casual inspection of the spectrum of the Douglas-fir stratified seed β-lectin (Fig. 27) serves to set it apart not only from that of the loblolly pine, but also from all the other β-lectin infrared spectra taken. The cause of this distinction is primarily the intensification of the amide absorptions. The amide I absorption (1660 cm^{-1}) dominates the spectrum with the amide II (1540 cm^{-1}) rivaling the generally dominant COH band (1080 cm^{-1}). In most of the other spectra (including that of the loblolly pine stratified seed β-lectin), the 1660 cm^{-1} band is only about as intense as the 1420 cm^{-1} band, with the 1540 cm^{-1} band appearing as a barely distinguishable bump in the trough between the two (Fig. 28). Secondary differences in the Douglas-fir spectrum consist of the dominance of the primary (1030 cm^{-1}) over the secondary (1080 cm^{-1}) COH stretching band and the splitting of the diffuse rocking absorption into two peaks at approximately 540 cm^{-1} and 640 cm^{-1}.

INTRASPECIES DIFFERENCES

Plant Parts

No significant differences are discernible in the infrared spectra from the β-lectins of sapling needles, stems, and roots of either species (Fig. 25 and 26).

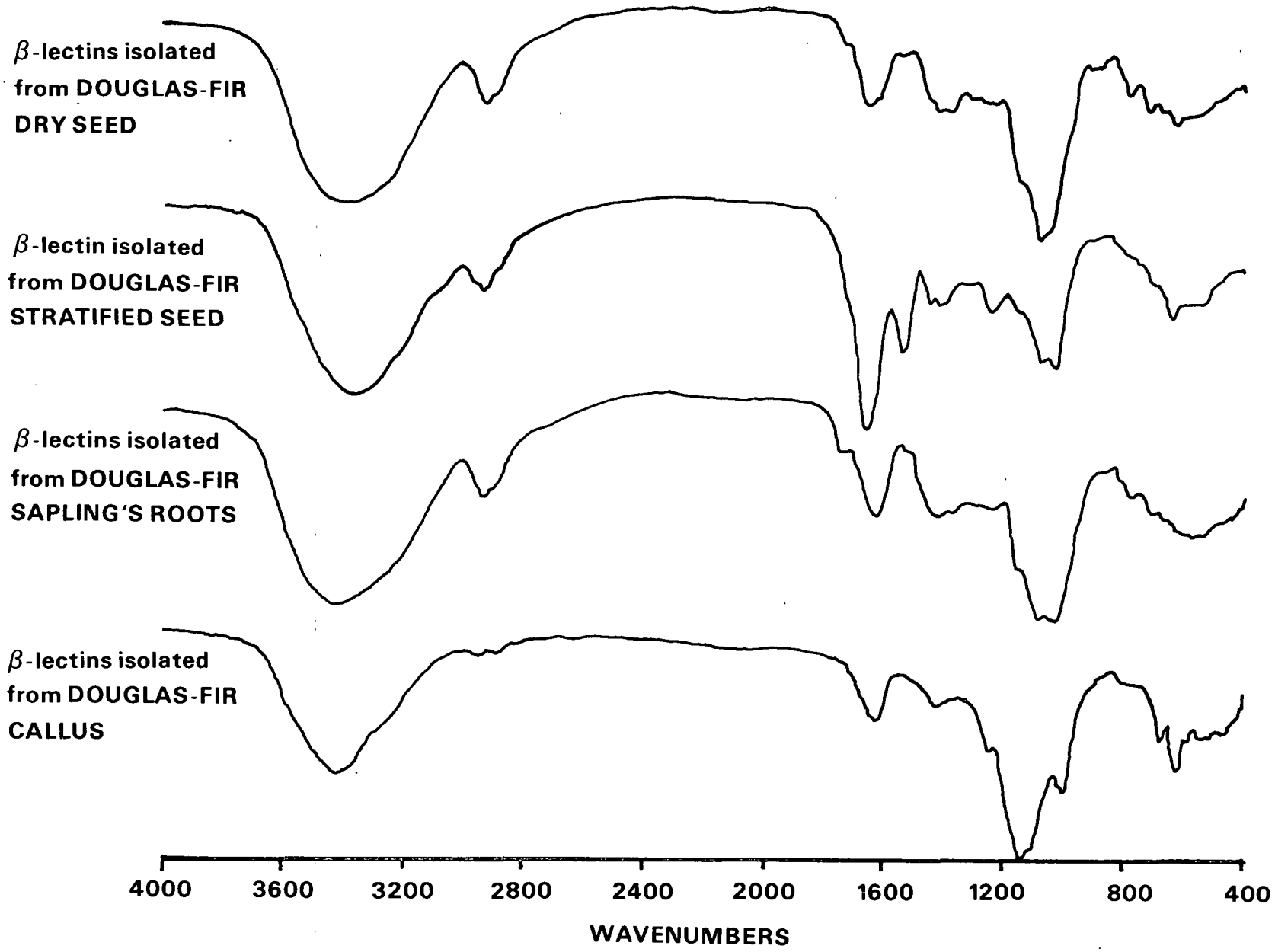


Figure 27. Intraspecies comparison: Douglas-fir (FTIR transmittance spectra of β-lectins from different developmental states).

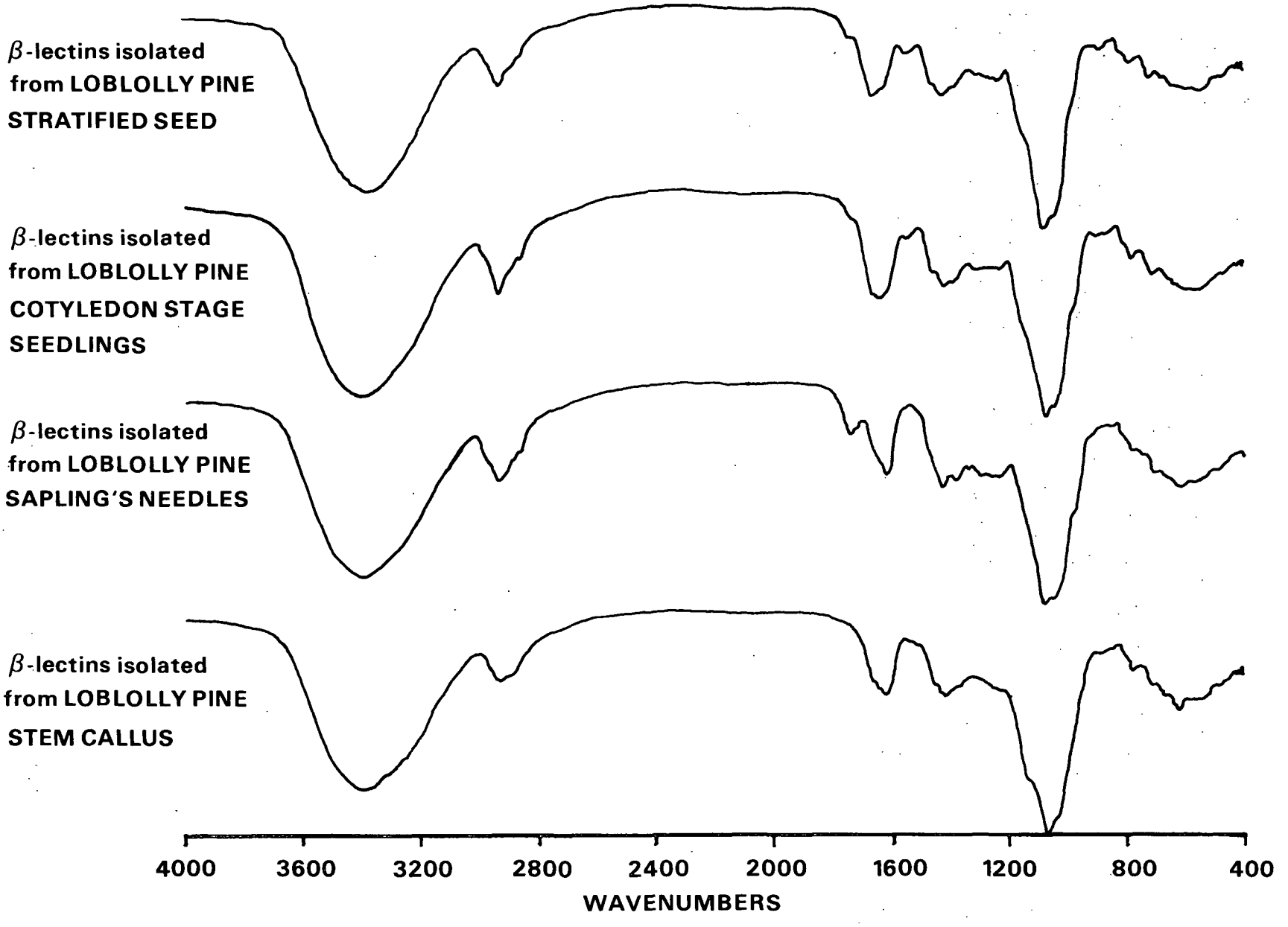


Figure 28. Intraspecies comparison: loblolly pine (FTIR transmittance spectra of β-lectins from different developmental states).

Plant Development

In a qualitative fashion the infrared spectra obtained confirm the results of the percent protein studies since the intensity of the amide peaks is proportional to the amount of protein in a sample of β-lectin. Thus, for the spectra of the Douglas-fir β-lectins, there was a dramatic change in the amide peaks going from the dry seed to the stratified seed samples (Fig. 27), another sharp drop going from the stratified seed to the sapling samples, with the callus sample showing a slight increase over the sapling sample (estimation via comparison of the 1620 cm^{-1} to the 1420 cm^{-1} absorption). Similarly, the spectra of the loblolly pine β-lectins demonstrate an increase in the 1620 cm^{-1} to the 1420 cm^{-1} bands going from the stratified seed to the cotyledon-seedling samples, and a drop going from the cotyledon to the sapling or calli samples (Fig. 28). These spectra thus corroborate the information obtained via the amino acid analysis estimation of the protein content of the β-lectin samples, as depicted in Fig. 13.

In addition to the amide I band variations, there are two other bands that seem sensitive to differences in the developmental state of the β-lectins, which may be related to structural variations in their carbohydrate moiety. The first band is that at 1030 cm^{-1} (the primary alcohol group's CO stretching), which seems to become more intense (relative to the 1080 cm^{-1} band) with development. The Douglas-fir stratified seed and sapling spectra show this intensification most clearly with the 1030 cm^{-1} absorption often being greater than that at 1080 cm^{-1}. In contrast to this, the Douglas-fir dry seed β-lectin spectrum shows the 1080 cm^{-1} peak clearly dominant, with only a slight shoulder at 1030 cm^{-1}. Such a trend can be noted in the loblolly pine spectra as well; the cotyledon-seedling and especially the sapling samples show the near equality of the bands, whereas the 1030 cm^{-1} band is considerably more attenuated in the stratified seed sample. For neither species was the 1030 cm^{-1} band intense for the calli samples (Fig. 29). The other band that

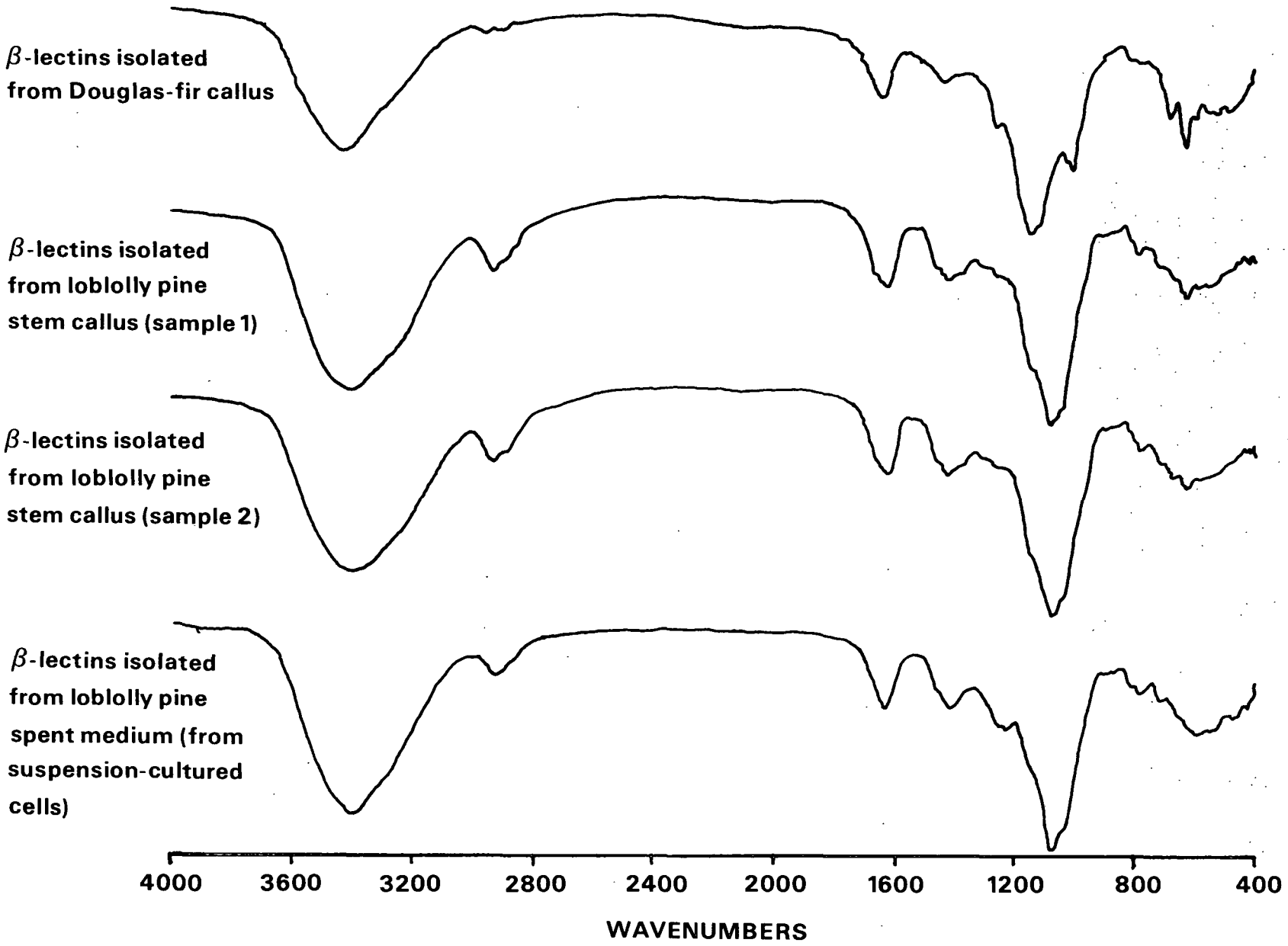


Figure 29. Interspecies comparison of the FTIR transmittance spectra of β-lectins from a common developmental state (callus).

seems to vary with development is that at 1730 cm^{-1}. Although the 1730 cm^{-1} band can be detected as a shoulder in the earlier stages of development (Douglas-fir dry seed and statified seed; loblolly pine stratified seed and cotyledon-seedling), it does not clearly emerge until the sapling stage in both species. In neither species is it detectable in any of the calli samples.

DISCUSSION

OCCURRENCE

Each of the null hypotheses (vide ante, Thesis Objective) concerning the absence of the β-lectins in conifer tissues has been invalidated. Thus, these compounds may indeed serve some important role in the life of the early plant. Furthermore, since all twenty null hypotheses were disproved, the probability that such a role is unique and narrowly localized in time is diminished.

On the contrary, this persistence of the β-lectins throughout the life and extent of the young plant places a constraint on any teleological hypothesis which is henceforth to be proposed for them.

COMMON CHARACTERISTICS

All of the β-lectins contained a protein component (1-30% by weight) bound to carbohydrate. Although the quantity of protein present varied, the constitution of the protein was virtually invariable. Acid hydrolyses of the β-lectin samples revealed 19 amino acids present in similar amounts from sample to sample regardless of the species, developmental state, or plant part. A comparison with published amino acid compositions from other gymnosperm species (Table XX, Appendix IV) shows that the similarity is not limited to the two species studied here (Fig. 30). In fact, all of the β-lectin amino acid compositions published to date resemble each other so closely as to suggest the existence of a common protein component controlled by a common ancestral gene. This hypothesis is supported by circumstantial evidence which indicates that it is a portion of the protein moiety which binds to the Yariv glucoside and thereby defines the compound as a β-lectin. The invariance of the β-lectins' electrofocusing banding pattern (at least in the pI 4.3-6.0 region) may be related to the constancy of protein composition, since Coomassie Blue staining bands tend to overlap with the strong Yariv glucoside staining bands.

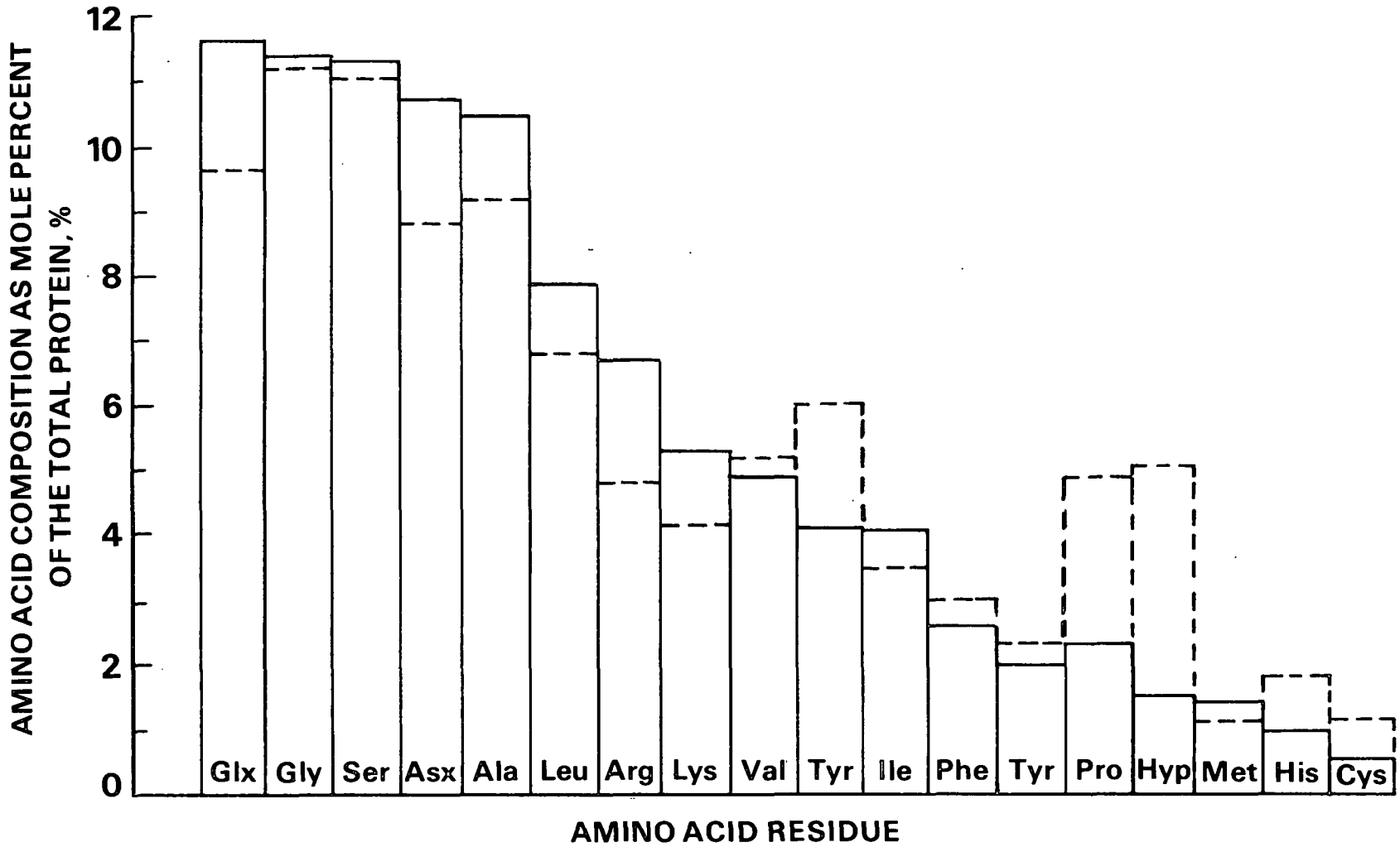


Figure 30. Comparison with amino acid analyses in the literature; solid lines represent the means of the 48 samples taken in this study; stippled lines represent the means of the 4 values reported in references (1,2). Data are given in Table XX, Appendix IV.

The carbohydrate portions of the β-lectins were not found to be as compositionally invariant as the protein moieties. Nonetheless, certain general characteristics are observable from sample to sample. Thus, in mole percentage the most abundant sugar was galactose (32-68%), on the average composing over half the neutral sugar fraction of the carbohydrate (Table XXXIV, Appendix VI). Since arabinose was the second most abundant sugar found (17-36%), these β-lectins can be considered as arabinogalactan proteins. However, the variable quantity of the less abundant sugars, especially glucose (4-34%), sets these Pinaceae β-lectins apart from simpler arabinogalactans such as those from Lolium multiflorum endosperm (11). In fact, the dry and stratified seed β-lectins have rhamnose to arabinose ratios approximately equal to those found in the side chains of Acacia senegal gum (gum arabic). Occasional aqueous insolubility after lyophilization and the implicated presence (by FTIR spectroscopy) of small amounts of uronic acids also indicate that the fine structure of the carbohydrate moieties of the β-lectins may have more in common with the relatively structurally complex arabinogalactan gum exudates than with the simpler known arabino-3,6-galactans (29,46).

Despite the variations in the relative amounts of the neutral sugar components and the varying amounts of protein, overall the β-lectins seem to have fundamentally similar structures. At least, that is what seems to be implied by the similarity in the infrared spectra and sedimentation coefficients of the various β-lectin samples studied. Not only do the spectra show the same absorptions from sample to sample, these absorptions also occur in approximately the same intensity. Since the protein is never the major component of the β-lectins, the stability of the nonamide absorptions seems to argue that the constituent carbohydrate groups (or combinations thereof) which give rise to the noted absorptions occur in approximately the same relative frequency from sample to sample. Again, since the protein moiety is not the major component of a β-lectin, the magnitude of the sedimentation coefficient is

likely to reflect the size and shape of the carbohydrate moiety. But the sedimentation coefficients do not exhibit large fluctuations in magnitude (range: 4.6-7.9). Hence, this line of reasoning also appears to converge on the probable existence of an essentially stable polysaccharide frame which can be found elaborated by the addition of further minor sugars and peptide strands.

INTERSPECIES DIFFERENCES

The most striking difference between the β-lectins of loblolly pine and Douglas-fir is that of their relative protein content. However, this difference is only striking for a portion of the developmental sequence studied (Fig. 1). The existence of this difference during the period of rapid growth, as determined by the amino acid analyses, is verified by the intensified amide bands in the infrared spectra of the samples (vide ante, Results, Fourier Transform Infrared Spectroscopy). The reason for such a species difference currently remains obscure.

Less obscure perhaps is the reason for the similarity in the electrofocusing patterns of the respective samples from each species. The amino acid compositions of the β-lectins from each species were not significantly different except for two residues. If the electrofocusing pattern is directly related to the protein constitution of the β-lectins, similarity of the banding patterns would be the expected outcome of an electrofocusing experiment.

Given the overall similarity of the amino acid compositions of the β-lectins from the two species, the two residues which were significantly different merit comment. These residues were hydroxyproline and aspartate (the sum of the asparagine and aspartic acid residues). Both hydroxyproline and asparagine are found as sites for the carbohydrate-peptide linkage in plant glycoproteins; in fact, they are probably the most commonly occurring sites for such linkages (41). Judging from the results of the present study and the use of the carbohydrate moieties of Acacia gums

as taxonomic markers (29), it appears that the carbohydrate moiety of the β-lectins may be the more recent part of the molecule in an evolutionary sense. If such were the case, it would not be surprising to find such variable peptide regions as do occur correlated with the variable carbohydrate. This line of inquiry may be worth pursuing, since O-glycosidic linkages through hydroxyproline have been reported for Phaseolus vulgaris β-lectins (44) and since hydroxyproline content has proven to be a useful marker for the evolutionary age of a species among the algae (76). The high hydroxyproline content of the Douglas-fir calli β-lectins might in part be explained by the observations that hydroxyproline content tends to be increased by stress situations and cold (76); callus tissue is produced via a wound response and the Douglas-fir calli (unlike the loblolly pine calli) were grown at a lower temperature during the dark phase of their circadian regime. With regard to the effect of cold on hydroxyproline content, it may be significant that Douglas-fir evolved in more northerly latitudes than did loblolly pine and that the gymnosperm with the highest level of hydroxyproline in Table XXI (Ginkgo biloba) survived only in the north of China (64).

N-glycosidic linkages through asparagine have not yet been reported in a β-lectin, although they were cited as being potentially present in the study on the linkage types present in Phaseolus vulgaris β-lectins (44). Two observations make the possibility of an N-glycosidic link through asparagine particularly attractive. The first of these is that the low amounts of hydroxyproline in the β-lectin (no hydroxyproline in the sapling samples which did have extensive carbohydrate moieties) seem inadequate to account for the persistent association of the peptide with the extensive carbohydrate moiety. The second is that loblolly pine, the species which absolute basis calculations shows to have the greater carbohydrate content, also is the species which is consistently found to be enriched with aspartate [ratios of grand averages for each parameter for Douglas-fir to loblolly pine (expressed as

percent): carbohydrate 80.9% and aspartate 77.3%]. The similarity in these ratios suggests that the enriched carbohydrate content of the loblolly pine may be due to a greater number of N-glycosyl linkages through asparagine in that species. This possibility is somewhat strengthened by considering the mutual decrease with time in the species ratios of the aspartate and sedimentation coefficient parameters (Fig. 31). Note that what has been plotted here is the decrease with time of the loblolly pine to Douglas-fir mean sedimentation coefficients and the decrease with time of the Douglas-fir to loblolly pine mean aspartate content.

The reason for interpreting the data in this manner lies in the apparently paradoxical behavior of the sedimentation coefficients. If the sedimentation coefficients reflect the shape and extent of the carbohydrate moiety of the β-lectin (as might be expected, since the protein is a quantitatively minor part of the proteoglycan), then the first interpretation one might give to Fig. 11 is that the Douglas-fir β-lectins are consistently richer in carbohydrate than those from loblolly pine. The carbohydrate analyses imply the opposite conclusion. The probable resolution of this apparent contradiction is that although the magnitude of a molecule's sedimentation coefficient is proportional to its molecular weight, it is also dependent on its partial specific volume and frictional coefficient. Thus, it is possible to have an increase in the partial specific volume and frictional coefficient offsetting the increase in mass to yield a sedimentation coefficient of lesser magnitude for a net increase in molecular weight. This could well be the case for these Pinaceae β-lectins and could explain the interspecies difference in the sedimentation coefficients. Thus, if the Douglas-fir β-lectin has the more highly branched structure implied by its neutral sugar analyses, it could be expected to have the more compact, spherical shape of gum arabic or amylopectin (29) as opposed to the slightly stiffer, bulkier shape of a polysaccharide with the majority of its galactose residues in the 1,3-galactan backbone. Such a difference in the carbohydrate structure could

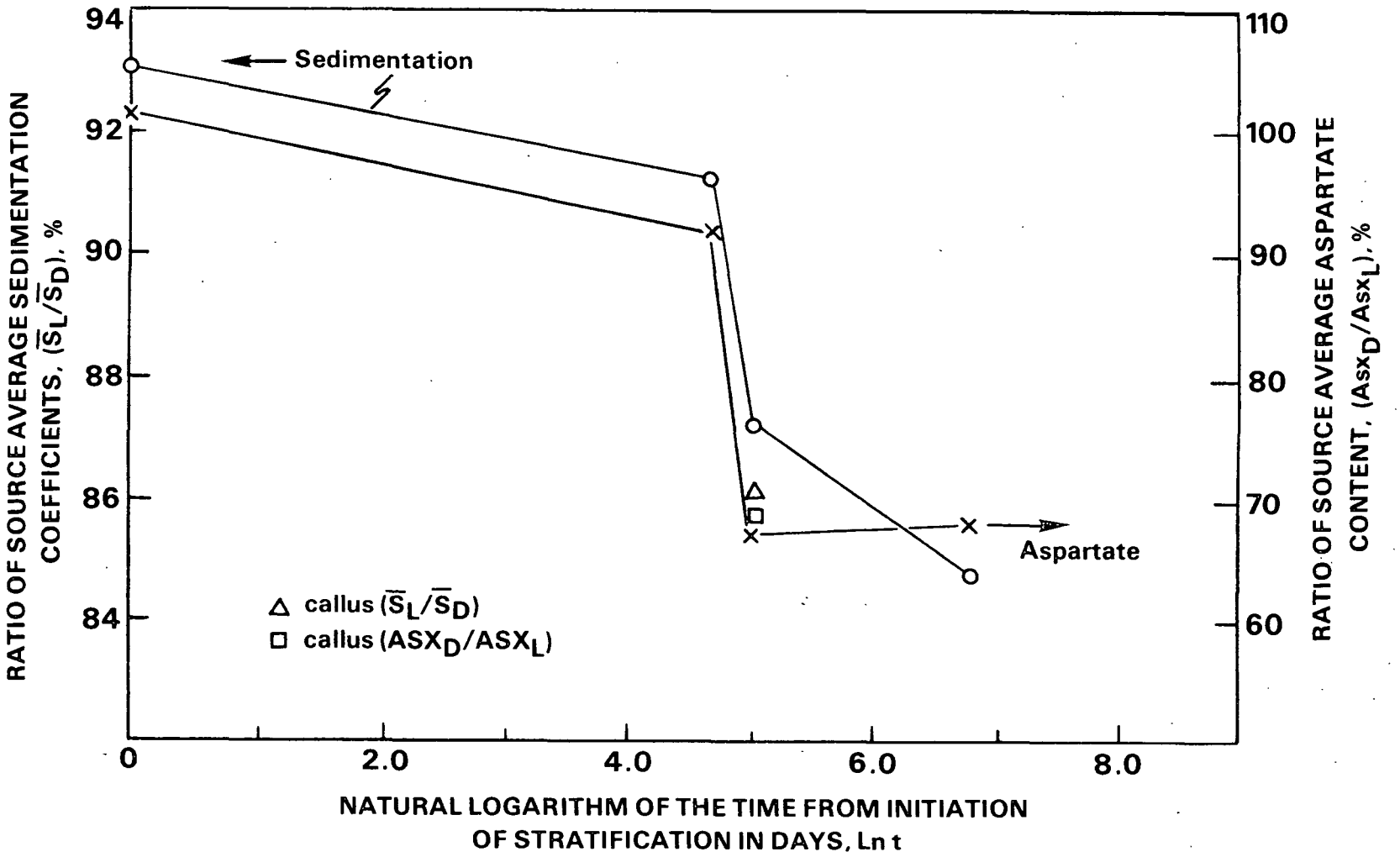


Figure 31. Comparison of the ratios of the species' β-lectin sedimentation coefficients and aspartate contents with time (for the callus, time is counted from callus initiation via explant).

possibly account for the nonamide absorption interspecies differences in the infrared spectra of the β-lectins.

INTRASPECIES DIFFERENCES

PLANT PARTS

Not a single one of the six parameters examined showed any significant differences in the β-lectins isolated from needles, stems, and roots from either species. Thus, although quantitative differences in the distribution of the β-lectins throughout the plant are indicated (by double diffusion studies), any qualitative differences which may exist appear to be very minor. Such similarity argues against any role for the β-lectins specific to chemical reactions uniquely localized in these plant parts. If this histochemical similarity in the β-lectins proves to be a general phenomenon, it will further delimit the range of teleological hypotheses which can be proposed for them.

PLANT DEVELOPMENT

As with the interspecies differences, the β-lectin parameter showing the greatest variation between developmental states is the percent protein. This is especially so for the Douglas-fir β-lectins, where the mean protein content rises by about 15% going from the dry seed to stratified seed states (Fig. 13). That such a change actually does take place is evidenced in the relative intensities of the amide absorptions in the FTIR spectra of the compounds (Fig. 23). That the pattern of this change is not only common to both species (Fig. 13), but often numerically identical (with respect to the data normalized to the dry seed basis of each species) is shown in Table V (Appendix III). The trend is for the weight percent protein of the β-lectin to double or triple that found in the dry seed upon germination and to persist at this level for at least the first two months of the seedling's growth.

Apparently, sometime after this period of rapid growth, there is a reduction in the percent protein of β-lectin, since the one- and four-year-old seedlings contained only about half the level found in the dry seed. This low value for the percent protein was maintained in the calli initiated from sapling material.

The lower values for the percent protein of the β-lectins from more mature tissue relative to the dry seed state is in agreement with an earlier report (2). In that report, the weight percent protein content of β-lectins from the leaves of 14 different species was found to range from 3 to 8% (compare with my data from the sapling material: 1-6%). It may be more than coincidental that, although the data were obtained from different species, the mean of the β-lectin weight percent protein from the leaves to the mean of the dry seed determinations was 0.4 (compare with my value for the pooled data of sapling to dry seed of 0.3, Table V).

Without precedent, however, is the report in this thesis of the rapid rise in β-lectin percent protein ensuing seed stratification. The reason for this rapid increase in the weight percent protein of the β-lectins is not currently known. It may be that this phenomenon is due to a flush of biosynthetic activity corresponding to the rapid growth of the plant during this period. If so, the β-lectins may result from assembling a number of smaller molecules (glycosylated peptides) into one large network. Subsequent elaboration of the carbohydrate moiety with enzymatic trimming of the peptide could account for the further alterations of β-lectins with the maturation of the plant. Such a scheme is not inconsistent with what is known about the biosynthesis of the pectins where whole arabinogalactan blocks seem to be incorporated into a larger structure whose external branches are then further decorated with arabinose and other neutral sugars (46). Protein trimming is implied by the results of β-lectin degradation studies (39,40) and a comparison of the amino acid composition of my protein-degraded Pinaceae β-lectins with the amino acid compositions of arabinogalactans isolated from the mature tissue of other species (73).

An attractive feature of such a model is that it explains how an increase in the amount of protein present does not result in a significant change in the relative proportions of the constituent amino acids. The presence of such a common unit protein is supported by the similar amino acid analyses and the similar electrofocusing banding patterns (evidently related to the former) of the β-lectin samples irrespective of the developmental state, plant part, or species from which they were isolated.

The mean sedimentation coefficient of the β-lectins follows a developmental trend similar to their mean weight percent protein; that is, there is a rise in the parameter's magnitude from the dry seed to the cotyledon seedling followed by a decrease in the sapling material. The parallel ranking of the data for the state means (normalized to the dry seed state) for the two species indicates that this developmental trend may be a general one. The correspondence of the developmental trends of these two parameters is not absolute, however, since the sedimentation coefficient departs from the percent proteins with respect to the callus state. Furthermore, this departure is rather drastic, since one parameter peaks in magnitude while the other reaches its near minimum value. Because of this departure and the lack of a significant interaction term in the two-way analysis of variance on the sedimentation coefficient (compare Fig. 11 and 12), it does not appear that these two parameters can be directly related to each other. The successive changes in the sedimentation coefficient with development do indicate a change in the overall shape and/or extent of the β-lectins. But since protein is not the major part of a β-lectin, it seems more likely to expect changes in the sedimentation coefficient to correspond to structural changes in the carbohydrate moiety. If this is the case, the similarity in developmental trend might be ascribed to tandem changes in the carbohydrate and the protein. That such is the case is implied by the striking intraspecies similarities in the neutral sugar analyses of the β-lectins from the cotyledon-seedling and callus states (Table XXXIII, Appendix VI) and the similarity

of their respective sedimentation coefficients (Fig. 11). In Fig. 17-19 the callus carbohydrate parameters were plotted against the time from callus initiation to harvest in order to emphasize their similarity to the cotyledon-seedling carbohydrate parameters. Not only is there a correspondence between the carbohydrate parameters and the sedimentation coefficients precisely where the correspondence breaks down for the sedimentation coefficients and the weight percent protein, but in general the two-way analysis of variance results are the same for the sedimentation coefficients and the carbohydrate parameters (Table XXXVI, Appendix VIII; significance for each of the main effects with their interaction term not significant).

The overall change in the carbohydrate moiety of the β-lectins with development is summarized in Fig. 18-20. As the plant develops, the trend is for the mole percent galactose (the most abundant neutral sugar) to fall as the mole percent of arabinose (the second most abundant neutral sugar) and glucose (the third most abundant neutral sugar) rises. Alternatively, this shift in the composition of the polysaccharide can be discerned by plotting time vs. the ratio of arabinose to galactose, or yet more inclusively of the nongalactose sugars to galactose (Fig. 19). Since the trend exists for both species, an overall estimate of it is obtained by plotting the pooled species data against time (Fig. 20). Plotting the data in this manner does more than simply indicate that there is a shift in β-lectin carbohydrate composition with development; it also indicates how this shift may be occurring. Carbohydrate structural studies on the β-lectins (31,32,35,47) have shown that nongalactose neutral sugars are found in side chains attached to a 1,3-β-D-galactan backbone. If the polysaccharide were enlarged by elaboration of the readily accessible side chains with more nongalactose neutral sugars, as occurs in the biosynthesis of pectins (49), the mole percent galactose of the polysaccharide would necessarily fall.

Such behavior is evident in Fig. 18-20. Moreover, two changes in the infrared spectra of the β-lectins with development also are indicative of successive carbohydrate side-chain elaboration. The changes referred to (vide ante, Results, FTIR) are the intensification of the primary (1030 cm^{-1}) over the secondary (1080 cm^{-1}) alcohol CO stretching absorptions and the gradual emergence of the uronic acid carbonyl stretching absorption at 1730 cm^{-1}. The intensification of the 1030 cm^{-1} absorption could occur as the ratio of primary to secondary alcohol groups in the β-lectin increased. Such a change might especially be looked for in the case of the 1,3-linked furanosyl pentoses, e.g., the 1,3-α-L-arabinofuranosyl residues reported to occur in β-lectin side chains (31). The greater 1030 cm^{-1} intensification observed in the Douglas-fir spectra of the stratified and sapling state samples may be related to the higher S/G (nongalactose sugar to galactose) ratio of this species (Table XXXIII, Appendix VI).

Unlike in the pectins, uronic acids are generally not major components of β-lectins (1,11,32). They evidently occur as end groups as they do in the uronic acids of the Larix laricina arabinogalactan (29,32). It is not surprising then to find the absorption characteristic of their presence most prominent in the sapling β-lectins of both species, the developmental state possessing the maximum S/G ratio for both species. In contrast to this, the absence of the 1730 cm^{-1} absorption from the callus samples is in accord with the absence of uronic acids in the β-lectins isolated from Lolium multiflorum suspension culture cells (11). In general, the smoother spectra of the β-lectin from the calli samples may imply that their carbohydrate moieties are structurally less varied (perhaps simpler) than those from the sapling samples. If such does prove to be the case, given the possible role of the carbohydrate moiety as an information carrier, the implications could merit pursuing.

CONCLUSIONS

The conclusions deducible from the data taken in the course of this study can be stated in terms of the two-part thesis objective. First, these two closely related species do contain the β-lectins in all of their principal plant parts throughout their period of early growth (from dry seed to sapling). Second, variations in various β-lectin parameters from these two species during their period of early growth indicate that the β-lectins are potentially useful developmental and taxonomic (but not histogenetic) markers.

More specifically, the 20 null hypotheses earlier established (vide ante, Proposal) have all been invalidated. In both loblolly pine and Douglas-fir, double-diffusion tests of plant extracts against Yariv glucoside revealed the presence of β-lectin in dry seeds; stratified seeds; cotyledon seedlings; the needles, stems, and roots from two-month-old seedlings and saplings; and callus. Since it is unlikely that the β-lectins are present only in those developmental states sampled, the reasonable inference is that they are present continuously throughout the life and extent of the young plant. It would not be surprising to find that this inference is also valid for the multitude of other plants found to contain β-lectins in their seeds (1,2,38). This widespread, generally nonlocalized occurrence of the β-lectins implies that they may indeed have an important function in a plant's life.

When the β-lectins were isolated from these various sources and characterized, they were found to be very similar to one another. All consisted of a minor protein component, nearly identical in composition but variable in quantity, and a major carbohydrate component (mainly arabinogalactan), which was more variable in composition. Despite the similarities, a comparison of the mean values of the parameters used to characterize these Pinaceae β-lectins revealed that they were not all

identical. On the contrary, correlations could be made between different structural parameters and the plant material from which the β-lectins were derived.

Species differences were discovered by comparison of the β-lectin parameter means from the successive developmental states of loblolly pine and Douglas-fir. Interspecies differences were found in the weight percent protein, in two of the 19 amino acids, in the neutral sugar composition, in the sedimentation coefficients, and in the infrared spectra of the β-lectins from these two representatives of the Pinaceae genera. Specifically, the Douglas-fir β-lectins were greater in protein content, sedimentation coefficient, hydroxyproline content, and percent nongalactose sugars, but less in aspartate content and overall neutral sugar content (especially in galactose) than the β-lectins of loblolly pine. Furthermore, the range in values for a given parameter was almost invariably greater for the Douglas-fir β-lectins. These differences are sufficient to establish the potential of the β-lectins as taxonomic markers.

No significant differences were detectable in the parameter means of β-lectins isolated from the needles, stems, and roots of the saplings from loblolly pine and Douglas-fir. These results indicate that the potential of the β-lectins as histogenetic markers is not great.

Developmental differences were discovered through a state-by-state comparison of the β-lectin parameter means of the two species. Developmental differences were found in the sedimentation coefficient, in the weight percent protein, in the neutral sugar composition, and in the infrared spectra of these conifer β-lectins. Specifically, the sedimentation coefficient and protein content of the β-lectins increased from the dry seed to cotyledon seedling and decreased thereafter, while the ratio of nongalactose sugars to galactose increased continually from the dry seed to sapling states (the changes in the infrared spectra tended to corroborate these

changes in the protein and carbohydrate moieties). It may be that these trends occur in the early growth of all β-lectin - containing spermatophytes. Whether or not these differences do indicate general trends, they are sufficient to establish the potential of the β-lectins as developmental markers.

RECOMMENDATIONS FOR FUTURE RESEARCH

STRUCTURAL STUDIES

Whatever the future goals of research on the β-lectins, further structural studies will probably be required to attain them. Certainly this is the case for some of the more promising research which can be envisioned, such as (1) the establishment of structure property correlations for appraising the feasibility of various β-lectin functional hypotheses, (2) the refinement of the use of the β-lectins as taxonomic and developmental markers, (3) the elucidation of the relationship of the β-lectins to other naturally occurring arabinogalactans, and (4) the utilization of the β-lectins as model compounds for investigating problems in the synthesis and degradation of plant glycoproteins.

The structural studies recommended can be grouped into two types. The first type is concerned with defining the overall structure and properties of the β-lectins and the second type with defining the fine structure of the protein and carbohydrate components of the β-lectins. Included in the first type of study might be molecular weight determinations by methods such as ultracentrifugation, gel chromatography, and osmotic pressure; viscosity determinations; and examination by x-ray diffraction and scanning electron microscopy. Included in the second type of study might be further electrofocusing studies and preparative electrophoresis to isolate the various subunits of the β-lectins. Such an approach could lead to identification of the Yariv glucoside binding portion of the β-lectin and a complete understanding of the bonding involved. N-terminal and amino acid sequence analyses of the purified components from the peptide-staining bands might be a means of testing Jermyn's tetrameric subunit hypothesis (5). Linkage analyses (particularly for the β-D-GlcNAc-Asn linkage) on the purified components from the bands sensitive to both the peptide and carbohydrate stains may reveal the locations as well as the quantity

and nature of the glycopeptide bonds. A better understanding of the fine structure of the carbohydrate moiety could be obtained by uronic acid and methylation analyses, acetolysis, partial acid hydrolysis, incubation with various glycosidases, and nuclear magnetic resonance spectroscopy.

A particular application for these carbohydrate structural studies is in extending the possibility explored in this thesis of using the β-lectins as taxonomic markers. Since the carbohydrate moiety is evidently the more recent part of the β-lectins in an evolutionary sense, it is the part most likely to reflect species diversification. This in fact was indicated by the present study but with an important qualification; not all developmental states reflect the carbohydrate differences to the same extent. Thus, a prior attempt to use the β-lectins as taxonomic markers (38) may have failed, since dry seeds were used as the β-lectin source material. The present study shows that, perhaps due to a form of molecular recapitulation, the dry seed state is the one with the least interspecies differences in any of the β-lectin parameters (including the neutral sugar analyses, whether on a mole percent or absolute basis). It is recommended that future studies to evaluate the chemotaxonomic potential of the β-lectins use a battery of carbohydrate structural determinations on β-lectins isolated from the tissue of fully mature plants of different species. Due to the near ubiquity of the β-lectins, such an approach may prove to be even more chemotaxonomically successful than previous studies on the arabinogalactan gum exudates of Acacia species (29).

BIOLOGICAL STUDIES

IN VIVO STUDIES

The use of β-lectins as developmental markers as initiated in the present study ought to be further investigated. The questions of the generality of the trends observed and the role of the β-lectins in the causality sequence of developmental

events can only be answered by further experimentation. Among the first studies which ought to be conducted are those to determine if the β-lectins are synthesized only at discrete periods in the plant's life and then modified, or if they are continuously synthesized and degraded, with the changes observed being due to the sensitivity of anabolic and catabolic systems to changing ambient conditions correlated with the different developmental states. This question (of molecular turnover) could probably be approached via the introduction of labeled precursors at appropriate stages in the plant's development. At least one representative each from the bryophytes (liverworts and mosses) or pteridophytes (ferns and fernallies), the gymnosperms, and the angiosperms would be attractive candidates for further developmental studies, since this would allow for confirmation of the trends in the gymnosperms and extension backward and forward in evolutionary time. Among the gymnosperms, Larix occidentalis could be an especially good choice for these studies, since a biosynthetic relationship between the β-lectins and the larch heartwood arabinogalactans might be revealed. Among the angiosperms, ryegrass (Lolium multiflorum), tobacco (Nicotiana tabacum), kidney bean (Phaseolus vulgaris), or sycamore (Acer pseudoplatanus) might be good choices, since β-lectins have been isolated from the media of suspension-cultured cells of these species and supplementary comparisons would be possible. Along with the developmental studies, a micrographic sequence of the cytological distribution of the β-lectins (as detected by staining with the Yariv glucoside) would be indispensable for a full interpretation of the molecular changes in terms of plant physiology.

Another line of inquiry which ought to be pursued is that concerned with the naturally occurring analog of the Yariv glucoside (22), the alcohol-soluble compound (apparently a flavonol glycoside) which inhibits the binding of the β-lectin to the Yariv glucoside. At the least, research on this precipitin inhibitor might clarify some aspects of the binding of the β-lectin to the Yariv compounds. It might even

be that a complete understanding of any physiological role for the β-lectins is impossible without an understanding of the close association of these two natural products. A starting point for an investigation of the inhibitor could be through comparison of the bonding of purified, characterized inhibitor to the structurally characterized β-lectins from the inhibitor's source tissue with the bonding of the inhibitor to structurally characterized β-lectins isolated from a noninhibitor containing source.

IN VITRO STUDIES

Reported in this study was the successful isolation of β-lectins from the calli of Douglas-fir and loblolly pine (77). They were also isolated from the spent growth media of suspension-cultured loblolly pine cells (34) and detected in the media of suspension-cultured wild carrot cells (73). Other researchers have isolated β-lectins from suspension-cultured cells of cherry (48,59), ryegrass (11), kidney bean (33), tobacco (32), and sycamore (5). Since the β-lectins occur so frequently in cultured cell systems, it is inevitable that more studies will be conducted on them using such systems. The results of the present study show that the β-lectins produced by tissue-cultured cells were not quite like any of those produced by tissue in the organized plant. Thus, however useful *in vitro* studies may prove to be in extending knowledge of the β-lectins, caution ought to be exercised in extrapolating their results back to the β-lectins found at different stages of the parent plant's development. In fact, one of the first β-lectin - oriented tissue-culture studies should be to clarify the nature of the structural differences that do exist between the β-lectins of *in vitro* and *in vivo* grown cells. Particularly helpful in this regard might be an examination of naturally occurring wound calli, both by micrographs of Yariv staining and characterization of β-lectin structural parameters. Such a study could potentially also reveal whether the β-lectins are involved in the early stages of cell wall formation or intercellular orientation.

Another application of suspension cultures which could be exploited here is the perturbation experiment. These experiments could determine if various *in vivo* grown β-lectins and companion inhibitors have any effect on the behavior of *in vitro* cultured cells.

Studies on the biosynthesis of the β-lectins have already begun using suspension cultures of ryegrass (50-54) and kidney bean (33). Not reported in this thesis was the discovery of a relationship between media sucrose concentration and the level of secreted β-lectins in suspension cultures of loblolly pine bud cells. It was also discovered that β-lectins are present in the jettisoned endosperm tissue of *Pinus taeda* and *Pinus sabiniana* (73). Given these two observations, the results of the *Lolium* biosynthesis studies, and the postulated function of Graham's sucrose synthetase (78,79), it is not unreasonable to test the hypothesis that the β-lectins act as storage polysaccharides. A suitable way to approach this problem might be to observe the fate (rate of catabolism, etc.) of suspension-cell cultured β-lectin relative to known storage polysaccharides in the presence of healthy and starved suspension-cultured cells.

GLOSSARY AND SPECIAL ABBREVIATIONS

AGP: arabinogalactan-protein

amino acid: a carboxylic acid containing an α-amino group, the L-forms of which constitute the monomeric units of proteins. According to international biochemical convention, the abbreviations used in this report represent the following amino acids:

Ala	alanine
Asx	aspartate, the sum of the asparagine (Asn) and the aspartic acid (Asp) residues
Arg	arginine
Cys	cysteine or 1/2 Cys (half-cystine)
His	histidine
Hyp	hydroxyproline
Ile	isoleucine
Glx	glutamate, the sum of the glutamine (Gln) and glutamic acid (Glu) residues
Gly	glycine
Leu	leucine
Lys	lysine
Met	methionine
Orn	ornithine
Phe	phenylalanine
Pro	proline
Ser	serine
Thr	threonine
Tyr	tyrosine
Val	valine

anomer: one of a pair of monosaccharides which differ from one another only in the position of the C1 hydroxyl or O-linked substituent above (β) or below (α) the cyclic ether ring (when the ring is in its standard Haworth projection with the oxygen atom in the upper right-hand corner of the hexagon).

AOV(2): analysis of variance, two-way. A statistical technique whereby the total variance of a process can be analyzed into its component factors (two in the case of the two-way AOV), the relative importance of which can then be assessed.

β-GLY: generic shorthand for a Yariv (65) glycosylphenylazo dye with the saccharide determinant in β linkage to the aglycone. Other Yariv compounds cited in the text are:

β-GLU: 1,3,5-tri-(p-β-D-glucopyranosyloxyphenylazo)-2,4,6-trihydroxybenzene
β-GAL: 1,3,5-tri-(p-β-D-galactopyranosyloxyphenylazo)-2,4,6-trihydroxybenzene
α-GAL: 1,3,5-tri-(p-α-D-galactopyranosyloxyphenylazo)-2,4,6-trihydroxybenzene
α-MAN: 1,3,5-tri-(p-α-D-mannopyranosyloxyphenylazo)-2,4,6-trihydroxybenzene

β-lectin: an arabinogalactan-protein that bonds specifically to the Yariv β-D-glycopyranosides.

callus: dedifferentiated plant cells propagated under aseptic conditions on an artificial growth medium in the laboratory.

configuration: spatial orientation of a molecule's atoms to one another (see also the Glossary entries for "D,L sugars" and "anomer").

cotyledon seedling: seedling at stage possessing cotyledons only.

D,L sugars: configuration assigned to a monosaccharide on the basis of whether the hydroxyl group on the lowest asymmetric carbon in a Fischer projection is to the right (D) or to the left (L).

development: ontogenetic growth

developmental state: a well characterized interval in the growth of a plant

Duncan's multiple range test: a statistical (multiple mean comparison) technique whereby the significance of a factor can be related to significant differences in the treatment levels of the factor's means.

FTIR: Fourier transform infrared spectroscopy. The spectrometer employs a helium-neon laser, microprocessor, and display screen and is enormously more sophisticated than conventional (dispersive) instruments.

furanose: a sugar existing as a five-membered ether-linked ring

G/A: the ratio of galactose to arabinose in an arabinogalactan

histogenetic: pertaining to the formation and development of tissues

IEF: isoelectric focusing (electrofocusing). An electrophoretic technique which separates the charged components of a substance by their isoelectric points.

interaction: the effect of one factor being dependent on the value of another factor

lectins: proteinaceous materials that noncovalently bond to specific carbohydrate residues

main effect: a quantity treated as an independent variable (factor) in an analysis of variance

marker: a molecule which is characteristic of a specific plant tissue or biochemical function

neutral sugars: a reducing sugar, one that reduces Fehling's, Benedict's, or Tollen's reagent; that is, a sugar that is capable of being oxidized by these reagents.

parameter: any of a set of physical properties whose values determine the characteristics or behavior of something.

PAS: periodic acid Schiff's reagent, a staining sequence used to detect carbohydrate

PBS: phosphate-buffered saline

pI: isoelectric point, the pH at which a molecule ceases to move in an electrofocusing trial, since its net surface charge is neutralized.

precipitin test: Ouchterlony double-diffusion test. A technique (borrowed from immunology) wherein the presence and bonding integrity of a pair of complementary molecules (usually an antigen and its antibody) is detected by the formation of a precipitate at the boundary of two diffusing fronts (of the solubilized molecules in a gel matrix).

pyranose: a sugar existing as a six-membered ether-linked ring

residue: monomeric unit of a polymer (such as a protein or polysaccharide)

sedimentation coefficient: the sedimentation rate for a unit centrifugal acceleration. For a given molecule in a given solvent at a given temperature, a characteristic constant.

sedimentation-velocity determination: an ultracentrifugation technique used to determine the sedimentation coefficient of a molecule. Solubilized molecules are subjected to high centrifugal forces and their boundary position in the centrifuge cell is recorded with time.

significance: statistical significance determined at the 5.0% level of probability

suspension-cultured cells: callus cells propagated in a liquid medium in vitro

S: one Svedberg unit = 1 S = 10^{-13} seconds, the units of the sedimentation coefficient. When the abbreviation "S" is not preceded by a scalar in this report, it is taken to mean "significant" in a statistical sense; "NS" means statistically nonsignificant.

taxonomic: said of a trait exhibited by members of one species which can be used to identify an individual as a member of that species.

UA: uronic acid, an aldose in which the hydroxymethyl group has been replaced by a carboxyl group

Yariv glycoside: a glycosylphenylazo dye of generic formula 1,3,5-tri-(p-glycosyloxyphenylazo)-2,4,6-trihydroxybenzene [cf. Glossary entry for β-GLY and Fig. 3 and 4 in "Materials & Methods"].

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APPENDIX I

CALLUS GROWTH MEDIA

TABLE I

MEDIA FOR DOUGLAS-FIR BUD TIP AND LOBLOLLY PINE STEM CALLI

Component	D[a] MS11	L[b] MSL1
NH_4NO_3	1650	1650
KNO_3	1900	1900
$CaCl_2 \cdot 2H_2O$	440	220
$MgSO_4 \cdot 7H_2O$	370	370
KH_2PO_4	170	340
H_3BO_3	6.2	6.2
$MnSO_4 \cdot H_2O$	16.9	16.9
$ZnSO_4 \cdot 7H_2O$	10.6	10.6
KI	0.83	0.83
$NaMoO_4 \cdot 2H_2O$	0.25	0.25
$CuSO_4 \cdot 5H_2O$	0.025	0.025
$CoCl_2 \cdot 6H_2O$	0.025	0.025
$FeSO_4 \cdot 7H_2O$	27.8	27.8
$Na_2 \cdot EDTA \cdot 2H_2O$	37.3	37.3
Nicotinic acid	0.5	0.5
Pyridoxine • HCl	0.1	0.1
Thiamine • HCl	0.1	0.1
Myo-inositol	100	100
β-napthoxyacetic acid[c]	0.1	0.1
N^6-benzylaminopurine	0.1	0.1
Sucrose	30,000	30,000
pH	5.8 ± 0.2	5.8 ± 0.2

[a]D = Douglas-fir bud tip callus growth medium.
[b]L = Loblolly pine stem callus growth medium. All concentrations as mg/L, ppm.
[c]Abbreviated as 2-NOAA.

APPENDIX II

ULTRACENTRIFUGATION DATA

TABLE II

PINACEAE β-LECTIN SEDIMENTATION COEFFICIENTS IN SVEDBERG UNITS

Source[a]		Sample Number 1	2	3	4	5	6	$\bar{X}$[b]	SD[b]
DS	D	5.87	5.48	5.13	4.93			5.35	0.41
	L	5.17	4.96	4.92	4.85			4.98	0.14
SS	D	6.60	6.10					6.35	0.35
	L	5.98	5.80	5.62				5.80	0.18
CT	D	7.09	6.65	6.62				6.79	0.26
	L	6.80	6.75	6.00	5.53	5.38	5.11	5.93	0.72
TM	D	6.90N[c]							
	L	5.08S[c]							
SP	D	6.61R[c]	6.45N	6.03R	5.97S	5.88N		6.19	0.32
	L	5.79S	5.53R	5.43S	5.35R	4.74N	4.59N	5.24	0.47
CL	D	7.94	7.62	7.22				7.59	0.36
	L	6.93	6.08					6.51	0.60

[a]The source code for the β-lectins from Douglas-fir (D) and loblolly pine (L) is as in Fig. 2, viz.

DS = dry seed
SS = stratified seed
CT = cotyledon seedling
TM = two-month-old seedling
SP = sapling
CL = callus

[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[c]N = Needles, S = stems, R = roots. Indicates the parts of the seedlings from which the β-lectins were isolated.

APPENDIX II (Continued)

TABLE III

INTERPRETATION OF SEDIMENTATION COEFFICIENTS

	DS[a]	SP	SS	CT	CL
$\overline{D}^{+}$	5.35	6.19	6.35	6.79	7.59
$\overline{L}^{+}$	4.98	5.24	5.80	5.93	6.51
$\overline{P}$	5.16[d]	5.67[c]	6.02[bc]	6.21[b]	7.16[a]
$\overline{D}^{*}$	1.00	1.16	1.19	1.27	1.42
$\overline{L}^{*}$	1.00	1.05	1.17	1.19	1.31
$\overline{P}^{*}$	1.00	1.10	1.17	1.20	1.39
$(\overline{D}-\overline{L})^{+}$	0.37	0.95	0.55	0.86	1.08
$(\overline{L}/\overline{D})^{+}$	93.1	84.6	91.3	87.3	85.8

[+]The source code is as in the preceding table. The other quantities are:

$\overline{D}$ = the source average of the sedimentation coefficients for the Douglas-fir (D) β-lectins as listed in Table II.

$\overline{L}$ = the source average of the sedimentation coefficients for the loblolly pine (L) β-lectins as listed in Table II.

$\overline{P}$ = the source average of the pooled sedimentation coefficients of the Pinaceae (pine and fir) β-lectins.
[abc]Duncan's Multiple Range Test; means followed by a common superscript are not significantly different.

$\overline{D}^{*}$ = the source average of the sedimentation coefficients for the Douglas-fir divided by the Douglas-fir dry seed average. Quantities marked by an asterisk are unitless, other quantities are in Svedberg units.

$\overline{L}^{*}$ = the loblolly pine source means normalized with respect to their dry seed state.

$\overline{P}^{*}$ = the pooled Pinaceae source means normalized with respect to their dry seed state.

$(\overline{D}-\overline{L})$ = the species difference of the source averages, pine from fir.

$(\overline{L}/\overline{D})$ = the species ratio of the source averages, pine to fir, expressed as percents.

APPENDIX III

PERCENT PROTEIN DATA

TABLE IV

PROTEIN AS WEIGHT PERCENT OF THE PINACEAE β-LECTIN SAMPLE

Source[a]		Sample Number 1	2	3	4	5	6	$\bar{X}$[b]	SD[b]
DS	D	10.6	9.5	8.3	6.7			8.8	1.7
	L	8.6	4.8	4.8				6.1	2.2
SS	D	25.9	21.1					23.5	3.4
	L	19.0	12.0	7.7	6.5	5.4		10.1	5.6
CT	D	30.2	27.1	13.9				23.7	8.6
	L	18.6	11.8	11.0	9.4	7.8	7.8	11.1	4.0
TM	D	29.8R[c]	17.4S[c]	16.6N[c]				21.3	7.4
	L	9.8N	9.5S	8.5R				9.3	0.7
SP	D	6.0R	4.0S	2.4N	2.0S	1.8N	1.6R	3.0	1.7
	L	2.9N	2.2R	1.3S	1.2N	1.0R	0.7S	1.6	0.8
CL	D	4.6	4.1	3.1	2.8			3.7	0.8
	L	4.6	2.9	2.4	1.2			2.8	1.4

[a]The source code for the β-lectins from Douglas-fir (D) and loblolly pine (L) is as in Fig. 2, viz.

DS = dry seed
SS = stratified seed
CT = cotyledon seedling
TM = two-month-old seedling
SP = sapling
CL = callus

[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.

[c]N = Needles, S = stems, R = roots. Indicates the parts of the seedlings from which the β-lectins were isolated.

APPENDIX III (Continued)

TABLE V

INTERPRETATION OF PROTEIN CONTENT

	SP[a]	CL	DS	TM	SS	CT
$\overline{D}^{+}$	3.0	3.7	8.8	21.5	23.5	23.7
$\overline{L}^{+}$	1.6	2.8	6.1	9.3	10.1	11.1
$\overline{P}^{+}$	2.3[c]	3.2[c]	7.6[b]	15.3[a]	13.9[a]	15.3[a]
$\overline{D}^{*}$	0.3	0.4	1.0	2.4	2.7	2.7
$\overline{L}^{*}$	0.3	0.5	1.0	1.5	1.7	1.8
$\overline{P}^{*}$	0.3	0.4	1.0	2.0	1.8	2.0
$(\overline{D}-\overline{L})^{+}$	1.4	0.9	1.7	9.3	11.1	10.1
$(\overline{L}/\overline{D})^{+}$	53.3	75.7	69.3	43.7	43.0	46.8

[+]The source code is as in the preceding table. The other quantities are:

$\overline{D}$ = the source average of the percent protein values for the Douglas-fir (D) β-lectins as listed in Table IV.

$\overline{L}$ = the source average of the percent protein values for the loblolly pine (L) β-lectins as listed in Table IV.

$\overline{P}$ = the source average of the pooled percent values for the Pinaceae (pine and fir) β-lectins.
[abc]Duncan's Multiple Range Test; means followed by a common superscript are not significantly different.

$\overline{D}^{*}$ = the source average of the percent protein values for the Douglas-fir divided by the Douglas-fir dry seed average. Values marked by an asterisk are unitless ratios, other quantities represent the protein quantity of the β-lectins expressed as weight percentages of the AGP sample.

$\overline{L}^{*}$ = the loblolly pine source means normalized with respect to their dry seed state.

$\overline{P}^{*}$ = the pooled Pinaceae source means normalized with respect to their dry seed state.

$(\overline{D}-\overline{L})$ = the species difference of the source averages, pine from fir.

$(\overline{L}/\overline{D})$ = the species ratio of the source averages, pine to fir, expressed as percents.

APPENDIX IV

AMINO ACID ANALYSES

TABLE VI

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

	Sample Number[a]				Species: Douglas-fir Source: Dry Seed (DS)	
Amino Acids	1	2	3	4	$\overline{X}$[b]	SD[b]
Glx	14.5	15.0	11.8	9.7	12.7	2.5
Gly	7.2	9.6	9.4	11.2	9.4	1.6
Ser	9.2	11.3	11.4	14.8	11.7	2.3
Asx	10.8	9.2	9.4	9.3	9.7	0.8
Ala	8.9	8.8	8.3	11.9	9.5	1.6
Leu	8.2	7.4	8.1	9.0	8.2	0.7
Arg	10.2	9.6	7.1	6.4	8.3	1.8
Lys	5.0	4.0	4.2	4.2	4.4	0.4
Val	6.0	5.2	6.1	6.6	6.0	0.6
Thr	2.0	3.4	3.3	3.3	3.0	0.7
Ile	4.5	4.1	4.3	3.5	4.1	0.4
Phe	3.3	3.6	4.1	3.5	3.6	0.3
Tyr	2.5	2.5	3.2	2.4	2.7	0.4
Pro	2.7	2.6	5.3	tr	2.7	2.2
Hyp	0.0	1.0	1.3	1.3	0.9	0.6
Met	1.6	0.7	2.1	1.6	1.5	0.6
Orn	0.6	tr	tr	tr	0.2	0.3
His	1.6	1.8	0.5	tr	1.0	0.9
1/2 Cys	1.4	0.0	0.1	1.3	0.7	0.7

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\overline{X}$ = Arithmetic average, SD = standard deviation.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE VII

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

Amino Acids	Sample Number[a]		Species: Douglas-fir Source: Stratified Seed (SS)	
	1	2	$\bar{x}$[b]	SD[b]
Glx	18.6	16.2	17.4	1.7
Gly	6.8	8.2	7.5	1.0
Ser	10.5	7.2	8.8	2.3
Asx	8.3	10.8	9.5	1.8
Ala	6.5	7.6	7.0	0.8
Leu	6.7	7.1	6.9	0.3
Arg	13.5	9.9	11.7	2.5
Lys	3.6	3.2	3.4	0.3
Val	3.9	6.5	5.2	1.8
Thr	2.2	4.2	3.2	1.4
Ile	3.5	4.2	3.9	0.5
Phe	2.4	3.3	2.8	0.6
Tyr	2.3	2.5	2.4	0.1
Pro	6.5	4.6	5.6	1.3
Hyp	1.1	0.0	0.6	0.8
Met	1.7	1.7	1.7	0.0
Orn	tr	0.2	0.1	0.1
His	tr	1.0	0.5	0.7
1/2 Cys	1.9	1.7	1.8	0.1

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE VIII

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

Amino Acids	Sample Number[a]			Species: Douglas-fir Source: Cotyledon Seedling (CT)	
	1	2	3	$\bar{X}$[b]	SD[b]
Glx	10.8	8.6	10.3	9.9	1.2
Gly	11.4	9.6	11.1	10.7	1.0
Ser	8.9	8.9	9.2	9.0	0.2
Asx	7.5	6.5	7.0	7.0	0.5
Ala	9.1	10.2	10.2	9.8	0.6
Leu	7.7	8.3	8.0	8.0	0.3
Arg	4.3	4.7	4.4	4.5	0.2
Lys	6.5	7.2	6.7	6.8	0.4
Val	6.3	6.9	6.4	6.5	0.3
Thr	7.2	7.3	7.0	7.2	0.2
Ile	4.6	4.7	4.4	4.6	0.2
Phe	3.3	3.7	3.5	3.5	0.2
Tyr	2.6	2.6	2.7	2.6	0.1
Pro	5.0	3.8	3.8	4.2	0.7
Hyp	1.8	3.4	1.6	2.3	1.0
Met	0.0	1.0	1.2	0.7	0.6
Orn	0.4	0.1	0.2	0.2	0.2
His	1.5	1.6	1.7	1.6	0.1
1/2 Cys	1.1	0.7	0.7	0.8	0.2

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE IX

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

	Sample Number[a]			Species: Douglas-fir Source: Two-Month Seedling (TM)	
Amino Acids	1N[c]	2S[c]	3R[c]	$\bar{X}$[b]	SD[b]
Glx	11.3	11.2	11.4	11.3	0.1
Gly	16.9	9.9	9.7	12.2	4.1
Ser	7.3	8.7	8.5	8.2	0.8
Asx	6.7	5.9	7.0	6.5	0.6
Ala	14.8	17.8	17.3	16.6	1.6
Leu	8.3	7.3	6.9	7.5	0.7
Arg	4.0	4.0	3.9	4.0	0.1
Lys	5.9	6.9	6.5	6.4	0.5
Val	6.7	6.2	6.0	6.3	0.4
Thr	2.4	5.6	5.8	4.6	1.9
Ile	4.0	4.6	4.3	4.3	0.3
Phe	3.2	3.1	3.0	3.1	0.1
Tyr	1.9	1.8	1.7	1.8	0.1
Pro	tr	2.8	3.0	1.9	1.7
Hyp	3.0	0.9	1.7	1.9	1.1
Met	1.9	1.6	1.4	1.6	0.3
Orn	tr	0.2	0.2	0.1	0.1
His	1.6	1.5	1.4	1.5	0.1
1/2 Cys	0.0	tr	0.2	0.1	0.1

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[c]N = Needles, S = stems, R = roots. Indicates the parts of the seedlings from which the β-lectins were isolated.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE X

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

Amino Acids	Sample Number[a] 1N[c]	2N	3S[c]	4S	5R[c]	6R	Species: Douglas-fir Source: Sapling (SP) $\bar{X}$[b]	SD[b]
Glx	11.4	14.7	10.2	16.8	15.6	13.0	13.6	2.5
Gly	18.7	15.1	16.7	15.1	10.1	16.4	15.4	2.9
Ser	12.4	15.5	21.4	18.4	12.2	11.6	15.3	4.0
Asx	12.4	12.4	12.1	10.6	14.0	12.5	12.3	1.1
Ala	7.3	8.1	8.4	8.6	9.5	11.6	8.9	1.5
Leu	7.3	5.8	8.4	3.7	7.8	5.8	6.5	1.7
Arg	21.8	8.9	13.0	1.5	5.0	4.8	9.2	7.3
Lys	4.1	3.5	4.7	2.2	4.4	2.9	3.6	1.0
Val	0.0	3.1	0.0	2.6	5.9	2.9	2.4	2.2
Thr	0.0	tr	0.1	4.5	1.2	5.8	1.9	2.6
Ile	3.1	3.1	3.7	2.4	5.0	3.4	3.5	0.9
Phe	0.1	1.5	0.1	1.3	3.5	tr	1.1	1.4
Tyr	0.1	1.5	0.1	2.2	4.2	tr	1.4	1.7
Pro	0.1	0.0	0.0	0.0	0.0	0.0	tr	0.0
Hyp	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Met	1.0	0.8	0.9	0.2	2.0	1.9	1.1	0.7
Orn	0.1	5.8	0.1	6.5	tr	7.2	3.3	3.5
His	0.1	0.0	0.1	3.5	tr	0.0	0.6	1.4
1/2 Cys	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[c]N = Needles, S = stems, R = roots. Indicates the parts of the seedlings from which the β-lectins were isolated.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE XI

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

Amino Acids	Sample Number[a]				Species: Douglas-fir Source: Callus (CL)	
	1[c]	2	3	4	$\bar{X}$[b]	SD[b]
Glx	10.2	7.4	7.3	8.0	8.2	1.4
Gly	10.5	10.3	9.7	10.2	10.2	0.3
Ser	9.2	14.3	13.8	12.3	12.4	2.3
Asx	8.8	6.7	6.8	7.0	7.3	1.0
Ala	8.3	14.1	12.9	13.5	12.2	2.6
Leu	6.5	6.7	6.6	7.2	6.8	0.3
Arg	4.9	2.7	3.6	2.8	3.5	1.0
Lys	5.5	4.2	5.6	5.0	5.1	0.6
Val	4.8	5.1	5.1	5.3	5.1	0.2
Thr	4.7	7.1	7.8	6.8	6.6	1.3
Ile	2.9	3.3	3.4	3.8	3.4	0.4
Phe	2.8	2.7	2.7	2.8	2.8	0.1
Tyr	2.2	2.0	1.9	2.0	2.0	0.1
Pro	6.2	2.2	3.4	3.5	3.8	1.7
Hyp	7.4	8.5	8.3	7.5	7.9	0.6
Met	1.0	1.1	0.2	1.0	0.8	0.4
Orn	1.0	0.4	0.0	0.3	0.4	0.4
His	2.0	1.1	1.0	0.8	1.2	0.5
1/2 Cys	0.5	tr	0.0	tr	0.1	0.2

[a]Each analysis represents a single determination on separate preparations of β-lectin.

[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.

[c]This sample was isolated from hypocotyl callus from a five-week-old-seedling. The other three samples are from bud-tip callus of a 2.7-year-old seedling (vide ante, Plant Materials). Growth conditions were the same for all calli.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE XII

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

Amino Acids	Sample Number[a]			Species: Loblolly Pine Source: Dry Seed (DS)	
	1	2	3	$\bar{X}$[b]	SD[b]
Glx	16.5	11.2	10.8	12.8	3.2
Gly	8.1	9.9	8.7	8.9	0.9
Ser	10.6	13.2	11.3	11.7	1.3
Asx	10.0	9.9	8.6	9.5	0.8
Ala	8.2	9.2	7.9	8.4	0.7
Leu	7.3	8.8	8.8	8.3	0.9
Arg	11.7	8.4	6.6	8.9	2.6
Lys	3.7	4.5	4.0	4.1	0.4
Val	4.6	6.4	6.3	5.8	1.0
Thr	2.3	3.6	2.5	2.8	0.7
Ile	3.1	3.9	4.8	3.9	0.9
Phe	2.3	3.7	4.3	3.4	1.0
Tyr	2.2	2.5	2.8	2.5	0.3
Pro	3.2	0.0	8.5	3.9	4.3
Hyp	0.0	1.2	0.8	0.7	0.6
Met	1.7	1.4	2.2	1.8	0.4
Orn	0.7	tr	tr	0.2	0.4
His	1.3	tr	1.2	0.8	0.7
1/2 Cys	2.6	2.2	tr	1.6	1.4

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE XIII

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

Amino Acids	Sample Number[a]					Species: Loblolly Pine Source: Stratified Seed (SS)	
	1	2	3	4	5	$\bar{X}$[b]	SD[b]
Glx	17.6	16.5	18.6	11.3	12.3	15.3	3.3
Gly	8.7	10.5	6.3	11.8	8.8	9.2	2.1
Ser	7.1	4.1	7.9	8.6	12.7	8.1	3.1
Asx	11.6	13.2	8.5	9.8	8.4	10.3	2.1
Ala	9.7	10.3	7.0	10.8	8.4	9.2	1.5
Leu	6.9	8.2	6.2	9.6	8.8	7.9	1.4
Arg	14.2	9.9	14.2	8.3	8.8	11.1	2.9
Lys	3.4	5.0	2.1	7.3	4.4	4.4	1.9
Val	4.3	5.8	4.7	6.3	5.6	5.3	0.8
Thr	6.7	3.7	4.1	tr	2.6	3.4	2.4
Ile	2.8	3.9	2.9	4.8	4.0	3.7	0.8
Phe	2.0	2.9	1.8	3.8	3.2	2.7	0.8
Tyr	2.0	2.5	2.4	2.8	2.8	2.5	0.3
Pro	tr	tr	6.3	tr	4.0	2.1	2.9
Hyp	0.0	0.0	0.6	1.3	1.2	0.6	0.6
Met	1.4	2.1	1.9	1.8	1.9	1.8	0.3
Orn	tr	tr	0.1	tr	tr	tr	0.0
His	1.4	1.4	0.9	tr	0.0	0.7	0.7
1/2 Cys	tr	tr	3.4	1.8	1.8	1.4	1.4

[a]Each analysis represents a single determination on separate preparations of β-lectin.

[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE XIV

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

Species: Loblolly Pine
Source: Cotyledon Seedling (CT)

Amino Acids	Sample Number[a] 1	2	3	4	5	6	$\bar{X}$[b]	SD[b]
Glx	11.1	10.6	10.9	9.1	8.0	8.0	9.6	1.4
Gly	11.5	10.9	11.1	9.1	9.8	9.8	10.4	0.9
Ser	9.7	8.9	10.4	16.7	11.3	9.4	11.1	2.9
Asx	7.4	11.2	7.0	13.1	12.2	11.4	10.4	2.6
Ala	12.1	11.1	10.2	10.5	9.8	9.3	10.5	1.0
Leu	7.3	7.5	7.6	8.5	7.8	7.2	7.7	0.5
Arg	3.7	4.6	5.0	5.4	5.1	5.0	4.8	0.6
Lys	6.2	5.8	5.6	7.5	7.5	6.6	6.5	0.8
Val	5.8	6.4	6.4	7.8	6.8	6.1	6.6	0.7
Thr	7.0	5.6	7.2	tr	3.8	6.6	5.0	2.8
Ile	3.8	4.2	4.1	5.1	4.8	4.4	4.4	0.5
Phe	2.9	3.2	3.1	3.0	3.0	2.9	3.0	0.1
Tyr	1.8	2.3	2.3	2.4	2.5	2.3	2.3	0.2
Pro	3.8	4.8	3.2	tr	0.0	3.9	2.6	2.1
Hyp	3.4	tr	2.7	0.0	0.0	0.0	1.0	1.6
Met	0.3	0.9	0.0	1.8	1.7	1.6	1.1	0.8
Orn	0.3	0.2	0.4	tr	3.6	2.8	1.2	1.6
His	1.1	1.2	1.2	tr	2.2	1.9	1.3	0.8
1/2 Cys	0.9	0.7	1.4	0.0	0.1	0.8	0.7	0.5

[a]Each analysis represents a single determination on separate preparations of β-lectin.

[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE XV

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

Amino Acids	Sample Number[a] 1N[c]	2S[c]	3R[c]	Species: Loblolly Pine Source: Two-Month Seedling (TM) $\bar{X}$[b]	SD[b]
Glx	10.2	12.6	12.4	11.7	1.3
Gly	11.1	6.5	6.7	8.1	2.6
Ser	6.6	8.9	8.8	8.1	1.3
Asx	11.9	7.2	8.2	9.1	2.5
Ala	10.9	10.3	10.8	10.7	0.3
Leu	9.0	8.2	7.6	8.3	0.7
Arg	4.4	5.8	5.1	5.1	0.7
Lys	4.9	6.3	5.8	5.7	0.7
Val	6.8	6.1	5.5	6.1	0.7
Thr	6.1	6.8	6.5	6.5	0.4
Ile	4.8	4.4	3.6	4.3	0.6
Phe	4.2	4.5	4.6	4.4	0.2
Tyr	2.3	3.0	3.0	2.8	0.4
Pro	3.7	3.8	3.3	3.6	0.3
Hyp	0.0	2.1	3.1	1.7	1.6
Met	1.4	1.5	1.5	1.5	0.1
Orn	tr	tr	0.4	0.1	0.2
His	1.7	2.0	2.1	1.9	0.2
1/2 Cys	tr	tr	0.8	0.3	0.5

[a]Each analysis represents a single determination on separate preparations of β-lectin.

[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.

[c]N = Needles, S = stems, R = roots. Indicates the parts of the seedlings from which the β-lectins were isolated.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE XVI

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

Amino Acids	Sample Number[a] 1N[c]	2N	3S[c]	4S	5R[c]	6R	Species: Loblolly Pine Source: Sapling $\bar{X}$[b]	SD[b]
Glx	12.8	18.7	6.5	3.5	10.4	8.9	10.1	5.3
Gly	13.8	14.1	19.5	15.4	9.8	18.8	15.2	3.6
Ser	10.4	18.6	10.8	7.6	15.9	11.8	12.5	4.0
Asx	13.8	16.9	21.6	22.5	17.4	16.8	18.2	3.3
Ala	8.7	6.4	10.8	11.8	8.1	13.8	9.9	2.7
Leu	8.0	7.2	10.8	11.1	11.9	8.9	9.7	1.9
Arg	5.5	0.1	6.5	6.3	8.8	3.0	5.0	3.0
Lys	3.8	4.0	6.5	7.0	8.8	5.9	6.0	1.9
Val	4.8	0.1	0.2	0.1	0.0	0.1	0.9	1.9
Thr	4.2	0.1	0.2	0.1	0.1	4.9	1.6	2.3
Ile	4.2	3.6	4.3	4.7	5.3	3.9	4.3	0.6
Phe	2.4	0.0	0.0	0.0	0.2	0.1	0.5	1.0
Tyr	2.1	0.0	0.0	0.0	0.2	0.0	0.4	0.8
Pro	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hyp	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Met	2.1	0.8	2.2	1.8	3.0	3.0	2.2	0.8
Orn	3.5	9.5	0.0	8.0	0.1	0.1	3.5	4.3
His	0.0	0.0	0.0	0.0	0.2	0.0	tr	0.1
1/2 Cys	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]Each analysis represents a single determination on separate preparations of β-lectin.

[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.

[c]N = Needles, S = stems, R = roots. Indicates the parts of the seedlings from which the β-lectins were isolated.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE XVII

PINACEAE β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

Amino Acids	Sample Number[a]			Species: Loblolly Pine Source: Callus (CL)	
	1	2	3	$\bar{X}$[b]	SD[b]
Glx	9.2	7.8	9.2	8.7	0.8
Gly	18.3	10.5	12.9	13.9	4.0
Ser	16.7	19.6	9.4	15.2	5.3
Asx	8.7	9.6	12.7	10.3	2.1
Ala	9.4	16.9	11.9	12.7	3.8
Leu	5.8	6.8	8.0	6.9	1.1
Arg	2.7	3.6	4.5	3.6	0.9
Lys	4.5	5.5	6.5	5.5	1.0
Val	2.5	5.0	6.2	4.6	1.9
Thr	5.2	4.6	4.2	4.6	0.5
Ile	2.0	3.2	4.5	3.2	1.3
Phe	2.0	2.3	4.2	2.8	1.2
Tyr	1.4	1.8	3.0	2.1	0.8
Pro	1.8	tr	tr	0.6	1.0
Hyp	2.6	0.5	0.7	1.3	1.2
Met	0.8	2.3	2.0	1.7	0.8
Orn	3.2	tr	tr	1.1	1.8
His	2.8	0.0	tr	0.9	1.6
1/2 Cys	0.0	0.0	0.0	0.0	0.0

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.

Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE XVIII

AMINO ACID COMPOSITION AS MOLE PERCENT OF THE TOTAL PROTEIN: SOURCE AVERAGES[a]

Amino Acids		DS	SS	CT	TM	SP	CL	$\overline{X}^b$	SD[b]
Glx	D	12.7	17.4	9.9	11.3	13.6	8.2	12.0	3.2
	L	12.8	15.3	9.6	11.7	10.1	8.7	11.3	3.7
Gly	D	9.4	7.5	10.7	12.2	15.4	10.2	11.5	3.3
	L	8.9	9.2	10.4	8.1	15.2	13.9	11.2	3.6
Ser	D	11.7	8.8	9.0	8.2	15.3	12.4	11.7	3.6
	L	11.7	8.1	11.1	8.1	12.5	15.2	11.0	3.8
Asx	D	9.7	9.5	7.0	6.5	12.3	7.3	9.2	2.4
	L	9.5	10.3	10.4	9.1	18.2	10.3	11.9	4.2
Ala	D	9.5	7.0	9.8	16.6	8.9	12.2	10.6	3.2
	L	8.4	9.2	10.5	10.7	9.9	12.7	10.2	2.1
Leu	D	8.2	6.9	8.0	7.5	6.5	6.8	7.2	1.1
	L	8.3	7.9	7.7	8.3	9.7	6.9	8.2	1.4
Arg	D	8.3	11.7	4.5	4.0	9.2	3.5	6.9	4.7
	L	8.9	11.1	4.8	5.1	5.0	3.6	6.4	3.4
Lys	D	4.4	3.4	6.8	6.4	3.6	5.1	4.8	1.4
	L	4.1	4.4	6.5	5.7	6.0	5.5	5.5	1.5
Val	D	6.0	5.2	6.5	6.3	2.4	5.1	4.9	2.0
	L	5.8	5.3	6.6	6.1	0.9	4.6	4.6	2.5
Thr	D	3.0	3.2	7.2	4.6	1.9	6.6	4.2	2.6
	L	2.8	3.4	5.0	6.5	1.6	4.6	3.8	2.5
Ile	D	4.1	3.9	4.6	4.3	3.5	3.4	3.8	0.7
	L	3.9	3.7	4.4	4.3	4.3	3.2	4.0	0.8
Phe	D	3.6	2.8	3.5	3.1	1.1	2.8	2.6	1.2
	L	3.4	2.7	3.0	4.4	0.5	2.8	2.6	1.5
Tyr	D	2.7	2.4	2.6	1.8	1.4	2.0	2.0	1.0
	L	2.5	2.5	2.3	2.8	0.4	2.1	1.9	1.0

See end of table for footnotes.

APPENDIX IV (Continued)

TABLE XVIII (Continued)

AMINO ACID COMPOSITION AS MOLE PERCENT OF THE TOTAL PROTEIN: SOURCE AVERAGES[a]

Amino Acid		DS	SS	CT	TM	SP	CL	$\bar{X}$[b]	SD[b]
Pro	D	2.7	5.6	4.2	1.9	tr	3.8	2.5	2.2
	L	3.9	2.1	2.6	3.6	0.0	0.6	1.9	2.4
Hyp	D	0.9	0.6	2.3	1.9	0.0	7.9	2.2	2.9
	L	0.7	0.6	1.0	1.7	0.0	1.3	0.8	1.1
Met	D	1.5	1.7	0.7	1.6	1.1	0.8	1.2	0.6
	L	1.8	1.8	1.1	1.5	2.2	1.7	1.7	0.7
Orn	D	0.2	0.1	0.2	0.1	3.3	0.4	1.1	2.2
	L	0.2	tr	1.2	0.1	3.5	1.1	1.3	2.5
His	D	1.0	0.5	1.6	1.5	0.6	1.2	1.0	0.9
	L	0.8	0.7	1.3	1.9	tr	0.9	0.9	0.9
1/2 Cys	D	0.7	1.8	0.8	0.1	0.0	0.1	0.4	0.6
	L	1.6	1.4	0.7	0.3	0.0	0.0	0.6	1.0

[a]Tabulated from the $\bar{X}$ columns of the preceding twelve tables, Table VI-XVII.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation. In this table, the average and its standard deviation are those of all the individual determinations made on each species, 22 for the Douglas-fir (D), and 26 for the loblolly pine (L). Trace (tr) values of a residue were averaged as zero. Trace amounts are those less than 0.05%.

APPENDIX IV (Continued)

TABLE XIX

DUNCAN MULTIPLE RANGE TESTS ON POOLED DATA

Amino Acids	DS+	SS	CT	TM	SP	CL	$\overline{X}$*	SD*
Glx	12.8^{b}	15.9^{a}	9.7^{bc}	11.5^{bc}	11.9^{b}	8.4^{c}	11.6^{a}	3.5
Gly	9.2^{bc}	8.7^{c}	10.5^{bc}	10.1^{bc}	15.3^{a}	11.8^{b}	11.4^{ab}	3.4
Ser	11.7^{ab}	8.3^{b}	10.4^{ab}	8.1^{b}	13.9^{a}	13.6^{a}	11.3^{ab}	3.7
Asx	9.6^{b}	10.1^{b}	9.3^{b}	7.8^{b}	15.2^{a}	8.6^{b}	10.7^{ab}	3.7
Ala	9.0^{b}	8.6^{b}	10.3^{b}	13.6^{a}	9.4^{b}	12.4^{a}	10.4^{b}	2.7
Leu	8.2	7.6	7.8	7.9	8.1	6.8	7.8^{c}	1.4
Arg	8.6^{ab}	11.3^{a}	4.7^{bc}	4.5^{bc}	7.1^{b}	3.5^{c}	6.6^{d}	4.0
Lys	4.2^{c}	4.1^{c}	6.6^{a}	6.0^{ab}	4.8^{c}	5.3^{bc}	5.2^{e}	1.5
Val	5.9^{ab}	5.3^{ab}	6.5^{a}	6.2^{ab}	1.6^{c}	4.9^{ab}	4.8^{ef}	2.3
Thr	2.9^{c}	3.4^{bc}	5.7^{a}	5.5^{ab}	1.8^{c}	5.8^{a}	4.0^{f}	2.5
Ile	4.0	3.7	4.5	4.3	3.9	3.3	4.0^{f}	0.7
Phe	3.5^{ab}	2.8^{ab}	3.2^{ab}	3.8^{a}	0.8^{c}	2.8^{b}	2.6^{g}	1.3
Tyr	2.6^{a}	2.5^{a}	2.4^{a}	2.3^{a}	0.9^{b}	2.6^{a}	2.0^{ghi}	1.0
Pro	3.2^{a}	3.1^{a}	3.1^{a}	2.8^{a}	0.0^{b}	2.4^{a}	2.2^{gh}	2.3
Hyp	0.8^{bd}	0.6^{cd}	1.4^{bc}	1.8^{b}	0.0^{d}	5.1^{a}	1.4^{hij}	2.2
Met	1.6	1.8	0.9	1.5	1.6	1.2	1.4^{hij}	0.7
Orn	0.2^{b}	0.0^{b}	0.9^{b}	0.1^{b}	3.4^{a}	0.7^{b}	1.2^{hij}	2.4
His	0.9^{ab}	0.7^{ab}	1.4^{a}	1.7^{a}	0.3^{b}	1.1^{ab}	0.9^{ij}	0.9
1/2 Cys	1.1^{ab}	1.5^{a}	0.7^{bc}	0.2^{cd}	0.0^{d}	0.1^{cd}	0.5^{j}	0.8

+Arithmetic mean of all the values (Douglas-fir and loblolly pine) for a developmental state as mole percent amino acid residue of the total β-lectin protein. The source code is as per Table II.

^{abcd}Duncan's Multiple Range Test (horizontal, for a residue across developmental states); means followed by a common superscript are not different at the 5% level of probability.

*$\overline{X}$ = Arithmetic mean, SD = standard deviation. In this case, all 48 determinations (on separate preparations of β-lectins) from Douglas-fir and loblolly pine have been averaged.

$^{ab\ldots j}$Duncan's Multiple Range Test (vertical, for the 19 residues across all states of the two species); means followed by a common superscript are not different at the 5% level of probability.

APPENDIX IV (Continued)

TABLE XX

GYMNOSPERM β-LECTIN AMINO ACID COMPOSITION
AS MOLE PERCENT OF THE TOTAL PROTEIN

Amino Acids	Sample Identification Number[a] 1	2	3	4	$\overline{X}$[b]	SD[b]	$\overline{X}$[c]	SD[b]
Glx	12.2	10.5	9.3	6.8	9.7	2.3	11.7	3.5
Gly	9.8	16.3	8.7	10.3	11.3	3.4	11.5	3.4
Ser	7.5	13.4	8.7	14.4	11.0	3.4	11.4	3.7
Asx	9.5	8.6	9.5	7.3	8.8	1.0	10.8	3.7
Ala	8.2	7.7	9.4	11.5	9.2	1.7	10.5	2.7
Leu	7.8	5.7	8.6	5.2	6.8	1.6	7.9	1.4
Arg	6.8	4.8	5.1	2.3	4.8	1.8	6.7	4.0
Lys	4.2	2.9	5.2	4.2	4.1	0.9	5.3	1.5
Val	6.4	3.8	6.2	4.5	5.2	1.3	4.9	2.3
Thr	4.6	5.7	6.4	7.4	6.0	1.2	4.1	2.5
Ile	4.3	2.9	4.0	2.8	3.5	0.8	4.0	0.7
Phe	4.3	2.9	3.7	2.4	3.3	0.8	2.6	1.3
Tyr	3.1	1.9	2.6	1.5	2.3	0.7	2.0	1.0
Pro	5.4	4.8	5.0	4.4	4.9	0.4	2.3	2.3
Hyp	1.7	5.7	2.1	11.1	5.1	4.3	1.5	2.2
Met	1.6	0.5	1.4	0.9	1.1	0.5	1.4	0.7
His	1.8	1.9	2.4	1.2	1.8	0.5	0.9	0.9
1/2 Cys	0.8	tr	1.7	1.8	1.1	0.8	0.5	0.8

[a]Sample classification according to Hutchinson (80-81).

	Class	Order	Family	Genus	Species
1	Gymnospermae	Coniferales	Araucariaceae	Araucaria	heterophylla
2	Gymnospermae	Coniferales	Taxodiaceae	Sequoia	sempervirens
3	Gymnospermae	Coniferales	Pinaceae	Pinus	contorta
4	Gymnospermae	Ginkgoales	Ginkgoaceae	Ginkgo	biloba

The data for Samples 1 to 3 are from dry seeds of the species (1). The data for Sample 4 are from the plant's leaves and have been normalized (omitting Orn, 0.5%) to comply with the data from (1).

[b]Arithmetic average ($\overline{X}$) and standard deviation (SD) of the four Gymnospermae β-lectin amino acid analyses in the literature (1,2).

[c]Arithmetic average ($\overline{X}$) and standard deviation (SD) of the 48 individual determinations reported in Tables VI-XVII. Ornithine (Orn) values have been omitted and the data accordingly normalized to comply with the data reported in (1).

APPENDIX V

β-LECTINS OF PLANT PARTS

TABLE XXI

COMPARISON OF AMINO ACID ANALYSES

Amino Acids		Needles[a]	Stems[a]	Roots[a]	$\overline{X}$[b]	SD[b]
Glx	D(3)	12.5,1.9	12.7,3.6	13.3,2.1		
	L(3)	13.9,4.3	7.5,4.6	10.6,1.8		
	P(6)	13.2,3.1	10.1,4.7	11.9,2.3	11.6	3.5
Gly	D(3)	16.9,1.8	13.9,3.5	12.1,3.8		
	L(3)	13.0,1.6	13.8,6.6	11.8,6.3		
	P(6)	12.5,2.6	13.8,4.8	11.9,4.6	11.4	3.4
Ser	D(3)	11.7,4.1	16.2,6.6	10.8,2.0		
	L(3)	11.9,6.1	9.1,1.6	12.2,3.6		
	P(6)	11.8,4.7	12.6,5.8	11.5,2.7	11.3	3.7
Asx	D(3)	10.5,3.3	9.5,3.2	11.2,3.7		
	L(3)	14.2,2.5	17.1,8.6	14.1,5.1		
	P(6)	12.3,3.3	13.3,7.1	12.6,4.3	10.7	3.7
Ala	D(3)	10.1,4.1	11.6,5.4	12.8,4.0		
	L(3)	8.7,2.2	11.0,0.8	10.9,2.8		
	P(6)	9.3,3.1	11.3,3.4	11.8,3.3	10.4	2.7
Leu	D(3)	7.1,1.3	6.3,2.4	6.8,1.0		
	L(3)	8.1,0.9	10.0,1.6	9.5,2.2		
	P(6)	7.6,1.1	8.2,2.7	8.1,2.1	7.8	1.4
Arg	D(3)	11.6,9.2	6.2,6.0	5.1,2.0		
	L(3)	3.3,2.8	6.2,0.4	5.6,2.9		
	P(6)	7.4,7.6	6.2,3.8	5.3,2.2	6.6	4.0
Lys	D(3)	4.5,1.2	4.6,2.3	4.6,1.8		
	L(3)	4.2,0.6	6.6,0.4	6.8,1.7		
	P(6)	4.4,0.9	5.6,1.9	5.7,2.0	5.2	1.5

See end of table for footnotes.

APPENDIX V (Continued)

TABLE XXI (Continued)

COMPARISON OF AMINO ACID ANALYSES

Amino Acids		Needles[a]	Stems[a]	Roots[a]	$\overline{X}$[b]	SD[b]
Val	D(3)	3.3,3.3	2.9,3.1	4.9,1.8		
	L(3)	3.9,3.4	2.1,3.4	1.9,3.1		
	P(6)	3.6,3.1	2.5,3.0	3.4,2.8	4.8	2.3
Thr	D(3)	1.9,3.3	3.4,2.9	4.3,2.6		
	L(3)	3.5,3.1	2.4,3.8	3.8,3.3		
	P(6)	2.7,3.0	2.9,3.1	4.0,2.7	4.0	2.5
Ile	D(3)	3.4,0.5	3.6,1.1	4.2,0.8		
	L(3)	4.2,0.6	4.5,0.2	4.3,0.9		
	P(6)	3.8,0.7	4.0,0.9	4.2,0.8	4.0	0.7
Phe	D(3)	1.6,1.5	1.5,1.5	2.2,1.9		
	L(3)	2.2,2.1	1.5,2.6	1.6,2.6		
	P(6)	1.9,1.7	1.5,1.9	1.9,2.0	2.6	1.3
Tyr	D(3)	1.2,0.9	1.4,1.1	2.0,2.1		
	L(3)	1.5,1.3	1.0,1.7	1.1,1.7		
	P(6)	1.3,1.0	1.2,1.3	1.5,1.8	2.0	1.0
Pro	D(3)	0.0,0.1	0.9,1.6	1.0,1.7		
	L(3)	1.2,2.1	1.3,2.2	1.1,1.9		
	P(6)	0.6,1.5	1.1,1.7	1.0,1.6	2.2	2.3
Hyp	D(3)	1.0,1.7	0.3,0.5	0.6,1.0		
	L(3)	0.0,0.0	0.7,1.2	1.0,1.8		
	P(6)	0.5,1.2	0.5,0.9	0.8,1.3	1.4	2.2
Met	D(3)	1.2,0.6	0.9,0.7	1.8,0.3		
	L(3)	1.4,0.6	1.8,0.3	2.5,0.9		
	P(6)	1.3,0.6	1.4,0.7	2.1,0.7	1.4	0.7

See end of table for footnotes.

APPENDIX V (Continued)

TABLE XXI (Continued)

COMPARISON OF AMINO ACID ANALYSES

Amino Acids		Needles[a]	Stems[a]	Roots[a]	$\overline{X}$[b]	SD[b]
Orn	D(3)	2.0,3.3	2.3,3.7	2.5,4.1		
	L(3)	4.3,4.8	2.7,4.6	0.2,0.2		
	P(6)	3.1,3.9	2.5,3.7	1.3,2.9	1.2	2.4
His	D(3)	0.6,0.9	1.7,1.7	0.5,0.8		
	L(3)	0.6,1.0	0.7,1.1	0.8,1.2		
	P(6)	0.6,0.8	1.2,1.4	0.6,0.9	0.9	0.9
1/2 Cys	D(3)	0.0,0.0	0.0,0.0	0.1,0.1		
	L(3)	0.0,0.0	0.0,0.0	0.3,0.5		
	P(6)	0.0,0.0	0.0,0.0	0.2,0.3	0.5	0.8

[a]Needles, stems, roots: the arithmetic mean and standard deviation ($\overline{X}$, SD) are given for each plant part for the Douglas-fir (D), loblolly pine (L) and pooled (P) data. The species means are from the two determinations on the sapling (Tables X and XVI) and one determination on the two-month seedling (Tables IX and XV) material.

[b]Arithmetic mean ($\overline{X}$) and standard deviation (SD) of the 48 individual determinations over all the developmental states of both species.

APPENDIX V (Continued)

TABLE XXII

COMPARISON OF MEANS

Parameter[a]	n[b]	Needles[b]	Stems[b]	Roots[b]	Student's t test[c] (N,S)	(N,R)	(S,R)
Sed. Coeff.	D(2)	6.17,0.40	5.97[d]	6.32,0.41	--	NS	--
	L(2)	4.67,0.11	5.61,0.25	5.44,0.13	S	S	NS
	P(4)	5.42,0.90	5.73[d],0.27	5.88,0.56	NS	NS	NS
Protein, %	D(2)	2.1,0.4	3.0,1.4	3.8,3.1	NS	NS	NS
	L(2)	2.0,1.2	1.0,0.4	1.6,0.8	NS	NS	NS
	P(4)	2.1,0.7	2.0,1.4	2.7,2.2	NS	NS	NS
Asx, %	D(3)	10.5,3.3	9.5,3.2	11.2,3.7	NS	NS	NS
	L(3)	14.2,2.5	17.1,8.6	14.1,5.1	NS	NS	NS
	P(6)	12.3,3.3	13.3,7.1	12.6,4.3	NS	NS	NS
Hyp, %	D(3)	1.0,1.7	0.3,0.5	0.6,1.0	NS	NS	NS
	L(3)	0.0,0.0	0.7,1.2	1.0,1.8	NS	NS	NS
	P(6)	0.5,1.2	0.5,0.9	0.8,1.3	NS	NS	NS
Gal, %	D(2)	47.2,1.8	40.8[d]	35.3,4.3	--	S	--
	L(2)	45.6,4.4	51.8,0.3	51.5[d]	NS	--	--
	P(4)	46.4,2.9	48.2[d],6.4	40.7[d],9.8	NS	NS	NS
Nongal, %	D(2)	52.8,1.8	59.2[d]	64.7,4.3	--	S	--
	L(2)	54.4,4.4	48.2,0.3	48.5[d]	NS	--	--
	P(4)	53.6,2.9	51.8[d],6.4	59.3[d],9.8	NS	NS	NS
S/G	D(2)	1.12,0.08	1.45[d]	1.85,0.35	--	NS	--
	L(2)	1.20,0.21	0.93,0.01	0.94[d]	NS	--	--
	P(4)	1.16,0.14	1.10[d],0.30	1.55[d],0.58	NS	NS	NS

See following page for footnotes.

APPENDIX V (Continued)

TABLE XXII (Continued)

COMPARISON OF MEANS

[a]Parameters characterized:
Sed. Coeff. = sedimentation coefficient in Svedberg units.
Protein, % = protein as weight percent of the β-lectin sample.
Asx, % = aspartate content as mole percent of the total protein moiety.
Hyp, % = hydroxyproline content as mole percent of the total protein moiety.
Gal, % = galactose content as mole percent of the total reducible carbohydrate.
Nongal, % = the sum of the nongalactose sugars (arabinose, glucose, rhamnose, mannose, xylose, fucose and ribose) as mole percent of the total reducible carbohydrate.
S/G = the ratio of nongalactose sugars to galactose (nongal, %/gal, %).

[b]Column headings:
n = the number of observations (single determinations on separate preparations of β-lectin) on the parameter for Douglas-fir (D), loblolly pine (L) and the pooled (P) species.
Needles, stems, roots: the arithmetic mean and standard deviation ($\overline{X}$,SD) are given for each plant part.

[c]Student's t test: comparison of the means for significance (S) or nonsignificance (NS) at the 5% level of probability, the pairings indicate which means are being compared.
(N,S) = needles' mean compared with stems' mean.
(N,R) = needles' mean compared with roots' mean.
(S,R) = stems' mean compared with roots' mean.
A dash indicates that no test was possible due to insufficient data.

[d]Case of less than the denoted n of observations.

APPENDIX VI

CARBOHYDRATE ANALYSES

TABLE XXIII

PINACEAE β-LECTIN NEUTRAL SUGAR COMPOSITION
AS MOLE PERCENT OF THE TOTAL NEUTRAL SUGARS

	Sample Number[a]			Species: Douglas-fir Source: Dry Seed (DS)	
Monosaccharide	1	2	3	$\bar{X}$[b]	SD[b]
Galactose	66.1	67.4	56.7	63.4	5.8
Arabinose	22.8	23.7	25.9	24.2	1.6
Glucose	4.6	4.4	4.1	4.4	0.2
Rhamnose	5.5	3.0	12.2	6.9	4.8
Mannose	0.0	0.6	0.7	0.4	0.4
Xylose	1.0	0.9	0.4	0.8	0.3
Ribose	0.0	0.0	0.0	0.0	0.0
Fucose	0.0	0.0	0.0	0.0	0.0
(G+A)[c]	88.9	91.1	82.6	87.5	4.4
(G+A+Glc)[c]	93.5	95.5	86.7	91.9	4.6
S[c]	33.9	32.6	43.3	36.6	5.8
(G/A)[c]	2.90	2.84	2.18	2.64	0.40
(A/G)[c]	0.34	0.35	0.46	0.38	0.07
(S/G)[c]	0.51	0.48	0.76	0.58	0.15

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[c]These quantities are derived from the data in the column under which they appear.
(G+A) = galactose plus arabinose.
(G+A+Glc) = galactose plus arabinose plus glucose.
S = the sum of the nongalactose sugars.
(G/A) = galactose divided by arabinose.
(A/G) = arabinose divided by galactose.
(S/G) = summed nongalactose sugars divided by galactose.

APPENDIX VI (Continued)

TABLE XXIV

PINACEAE β-LECTIN NEUTRAL SUGAR COMPOSITION
AS MOLE PERCENT OF THE TOTAL NEUTRAL SUGARS

Monosaccharide	Sample Number[a]			Species: Douglas-fir Source: Stratified Seed (SS)	
	1	2	3	$\bar{X}$[b]	SD[b]
Galactose	43.3	58.3	50.0	50.5	7.5
Arabinose	21.9	23.2	17.2	20.8	3.2
Glucose	13.4	5.3	26.8	15.1	10.8
Rhamnose	8.0	11.7	0.0	6.6	6.0
Mannose	7.5	0.5	3.4	3.8	3.5
Xylose	5.9	1.0	2.6	3.2	2.5
Ribose	0.0	0.0	0.0	0.0	0.0
Fucose	0.0	0.0	0.0	0.0	0.0
(G+A)[c]	65.2	81.4	67.2	71.3	8.8
(G+A+Glc)[c]	78.6	86.8	94.0	86.4	7.7
S[c]	56.7	41.7	50.0	49.5	7.5
(G/A)[c]	1.98	2.52	2.90	2.47	0.46
(A/G)[c]	0.51	0.40	0.34	0.42	0.09
(S/G)[c]	1.31	0.72	1.00	1.01	0.29

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[c]These quantities are derived from the data in the column under which they appear.
(G+A) = galactose plus arabinose.
(G+A+Glc) = galactose plus arabinose plus glucose.
S = the sum of the nongalactose sugars.
(G/A) = galactose divided by arabinose.
(A/G) = arabinose divided by galactose.
(S/G) = summed nongalactose sugars divided by galactose.

APPENDIX VI (Continued)

TABLE XXV

PINACEAE β-LECTIN NEUTRAL SUGAR COMPOSITION
AS MOLE PERCENT OF THE TOTAL NEUTRAL SUGARS

	Sample Number[a]			Species: Douglas-fir Source: Cotyledon Seedling (CT)	
Monosaccharide	1	2	3	$\bar{X}$[b]	SD[b]
Galactose	50.3	50.0	40.4	46.9	5.6
Arabinose	35.0	32.9	26.1	31.3	4.6
Glucose	12.7	14.7	27.9	18.4	8.2
Rhamnose	0.0	0.0	0.0	0.0	0.0
Mannose	2.0	2.4	5.6	3.3	2.0
Xylose	0.0	0.0	0.0	0.0	0.0
Ribose	0.0	0.0	0.0	0.0	0.0
Fucose	0.0	0.0	0.0	0.0	0.0
(G+A)[c]	85.3	82.9	66.5	78.2	10.2
(G+A+Glc)[c]	98.0	97.6	94.4	96.7	2.0
S[c]	49.7	50.0	59.6	53.1	5.6
(G/A)[c]	1.44	1.52	1.55	1.50	0.06
(A/G)[c]	0.70	0.66	0.65	0.67	0.03
(S/G)[c]	0.99	1.00	1.47	1.15	0.27

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[c]These quantities are derived from the data in the column under which they appear.
(G+A) = galactose plus arabinose.
(G+A+Glc) = galactose plus arabinose plus glucose.
S = the sum of the nongalactose sugars.
(G/A) = galactose divided by arabinose.
(A/G) = arabinose divided by galactose.
(S/G) = summed nongalactose sugars divided by galactose.

APPENDIX VI (Continued)

TABLE XXVI

PINACEAE β-LECTIN NEUTRAL SUGAR COMPOSITION
AS MOLE PERCENT OF THE TOTAL NEUTRAL SUGARS

	Sample Number[a]					Species: Douglas-fir Source: Sapling (SP)	
Monosaccharide	1N[b]	2N	3S[b]	4S	5R[b]	$\bar{X}$[c]	SD[c]
Galactose	48.5	45.9	40.8	38.4	32.3	41.2	6.4
Arabinose	36.2	32.7	34.2	25.1	29.7	31.6	4.3
Glucose	10.0	13.5	20.3	29.5	33.9	21.4	10.2
Rhamnose	2.3	2.7	2.0	1.9	1.8	2.2	0.4
Mannose	1.3	3.4	1.1	2.6	1.8	2.0	0.9
Xylose	0.7	0.9	0.8	1.8	0.5	0.9	0.5
Ribose	0.3	0.0	0.0	0.0	0.0	0.1	0.1
Fucose	0.7	0.9	0.8	0.7	0.0	0.6	0.4
(G+A)[d]	84.7	78.6	75.0	63.5	62.0	72.8	9.8
(G+A+Glc)[d]	94.7	92.1	95.3	93.0	95.9	94.2	1.6
S[d]	51.5	54.1	59.2	61.6	67.7	58.8	6.4
(G/A)[d]	1.34	1.40	1.19	1.53	1.09	1.31	0.17
(A/G)[d]	0.75	0.71	0.84	0.65	0.92	0.77	0.11
(S/G)[d]	1.06	1.18	1.45	1.61	2.10	1.48	0.41

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]N = needles, S = stems, R = roots. Indicates the parts of the seedlings from which the β-lectins were isolated.
[c]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[d]These quantities are derived from the data in the column under which they appear.
(G+A) = galactose plus arabinose.
(G+A+Glc) = galactose plus arabinose plus glucose.
S = the sum of the nongalactose sugars.
(G/A) = galactose divided by arabinose.
(A/G) = arabinose divided by galactose.
(S/G) = summed nongalactose sugars divided by galactose.

APPENDIX VI (Continued)

TABLE XXVII

PINACEAE β-LECTIN NEUTRAL SUGAR COMPOSITION
AS MOLE PERCENT OF THE TOTAL NEUTRAL SUGARS

Monosaccharide	Sample Number[a]			Species: Douglas-fir Source: Callus (CL)	
	1	2	3	$\bar{X}$[b]	SD[b]
Galactose	45.3	48.7	48.8	47.6	2.0
Arabinose	35.0	36.3	35.6	35.6	0.7
Glucose	16.2	12.7	12.3	13.8	2.1
Rhamnose	0.0	0.0	0.0	0.0	0.0
Mannose	2.5	2.3	2.7	2.5	0.2
Xylose	1.0	0.0	0.6	0.5	0.5
Ribose	0.0	0.0	0.0	0.0	0.0
Fucose	0.0	0.0	0.0	0.0	0.0
(G+A)[c]	80.3	85.0	84.4	83.2	2.5
(G+A+Glc)[c]	96.5	97.7	96.7	97.0	0.6
S[c]	54.7	51.3	51.2	52.4	2.0
(G/A)[c]	1.30	1.34	1.37	1.34	0.03
(A/G)[c]	0.77	0.75	0.73	0.75	0.02
(S/G)[c]	1.21	1.05	1.05	1.10	0.09

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[c]These quantities are derived from the data in the column under which they appear.
(G+A) = galactose plus arabinose.
(G+A+Glc) = galactose plus arabinose plus glucose.
S = the sum of the nongalactose sugars.
(G/A) = galactose divided by arabinose.
(A/G) = arabinose divided by galactose.
(S/G) = summed nongalactose sugars divided by galactose.

APPENDIX VI (Continued)

TABLE XXVIII

PINACEAE β-LECTIN NEUTRAL SUGAR COMPOSITION
AS MOLE PERCENT OF THE TOTAL NEUTRAL SUGARS

Monosaccharide	Sample Number[a] 1	2	3	Species: Douglas-fir Source: Dry Seed (DS) $\bar{X}$[b]	SD[b]
Galactose	60.5	56.5	68.0	61.7	5.8
Arabinose	28.3	25.6	20.8	24.9	3.8
Glucose	4.5	5.9	8.6	6.3	2.1
Rhamnose	6.4	10.5	1.2	6.0	4.7
Mannose	0.0	1.0	0.7	0.6	0.5
Xylose	0.3	0.5	0.7	0.5	0.2
Ribose	0.0	0.0	0.0	0.0	0.0
Fucose	0.0	0.0	0.0	0.0	0.0
(G+A)[c]	88.8	82.1	88.8	86.6	3.9
(G+A+Glc)[c]	93.3	88.0	97.4	92.9	4.7
S[c]	39.5	43.5	32.0	38.3	5.8
(G/A)[c]	2.14	2.21	3.27	2.54	0.63
(A/G)[c]	0.47	0.45	0.31	0.41	0.09
(S/G)[c]	0.65	0.77	0.47	0.63	0.15

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[c]These quantities are derived from the data in the column under which they appear.
(G+A) = galactose plus arabinose.
(G+A+Glc) = galactose plus arabinose plus glucose.
S = the sum of the nongalactose sugars.
(G/A) = galactose divided by arabinose.
(A/G) = arabinose divided by galactose.
(S/G) = summed nongalactose sugars divided by galactose.

APPENDIX VI (Continued)

TABLE XXIX

PINACEAE β-LECTIN NEUTRAL SUGAR COMPOSITION
AS MOLE PERCENT OF THE TOTAL NEUTRAL SUGARS

Monosaccharide	Sample Number[a]			Species: Loblolly Pine Source: Stratified Seed (SS)	
	1	2	3	$\bar{X}$[b]	SD[b]
Galactose	57.7	56.0	59.6	57.8	1.8
Arabinose	26.3	26.8	27.4	26.8	0.5
Glucose	5.4	9.3	5.3	6.7	2.3
Rhamnose	7.3	5.5	6.6	6.5	0.9
Mannose	2.4	1.4	0.8	1.5	0.8
Xylose	0.8	1.0	0.3	0.7	0.4
Ribose	0.0	0.0	0.0	0.0	0.0
Fucose	0.0	0.0	0.0	0.0	0.0
(G+A)[c]	84.0	82.8	87.0	84.6	2.2
(G+A+Glc)[c]	89.4	92.1	92.3	91.3	1.6
S[c]	42.3	44.0	40.4	42.2	6.5
(G/A)[c]	2.19	2.09	2.17	2.15	0.05
(A/G)[c]	0.46	0.48	0.46	0.47	0.01
(S/G)[c]	0.73	0.79	0.68	0.73	0.05

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[c]These quantities are derived from the data in the column under which they appear.
(G+A) = galactose plus arabinose.
(G+A+Glc) = galactose plus arabinose plus glucose.
S = the sum of the nongalactose sugars.
(G/A) = galactose divided by arabinose.
(A/G) = arabinose divided by galactose.
(S/G) = summed nongalactose sugars divided by galactose.

APPENDIX VI (Continued)

TABLE XXX

PINACEAE β-LECTIN NEUTRAL SUGAR COMPOSITION
AS MOLE PERCENT OF THE TOTAL NEUTRAL SUGARS

	Sample Number[a]			Species: Loblolly Pine Source: Cotyledon Seedling (CS)	
Monosaccharide	1	2	3	$\bar{X}$[b]	SD[b]
Galactose	55.0	46.6	51.9	51.2	4.2
Arabinose	33.9	33.0	29.4	32.1	2.4
Glucose	5.8	14.0	11.2	10.3	4.2
Rhamnose	2.9	2.1	2.3	2.4	0.4
Mannose	1.2	2.7	4.4	2.8	1.6
Xylose	0.3	0.6	0.5	0.5	0.1
Ribose	0.9	0.0	0.0	0.3	0.5
Fucose	0.0	1.0	0.3	0.4	0.5
(G+A)[c]	88.9	79.6	81.3	83.3	4.9
(G+A+Glc)[c]	94.7	93.6	92.5	93.6	1.1
S[c]	45.0	53.4	48.1	48.8	4.2
(G/A)[c]	1.62	1.41	1.76	1.60	0.18
(A/G)[c]	0.62	0.71	0.57	0.63	0.07
(S/G)[c]	0.82	1.15	0.93	0.97	0.17

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[c]These quantities are derived from the data in the column under which they appear.
(G+A) = galactose plus arabinose.
(G+A+Glc) = galactose plus arabinose plus glucose.
S = the sum of the nongalactose sugars.
(G/A) = galactose divided by arabinose.
(A/G) = arabinose divided by galactose.
(S/G) = summed nongalactose sugars divided by galactose.

APPENDIX VI (Continued)

TABLE XXXI

PINACEAE β-LECTIN NEUTRAL SUGAR COMPOSITION
AS MOLE PERCENT OF THE TOTAL NEUTRAL SUGARS

	Sample Number[a]					Species: Loblolly Pine Source: Sapling (SP)	
Monosaccharide	1N[b]	2N	3S[b]	4S	5R[b]	$\bar{X}$[c]	SD[c]
Galactose	48.8	42.5	51.6	52.1	51.5	49.3	4.0
Arabinose	30.4	26.0	29.1	29.7	24.3	27.9	2.6
Glucose	11.1	24.0	12.1	11.3	15.5	14.8	5.4
Rhamnose	5.8	4.4	4.0	5.5	2.6	4.5	1.3
Mannose	2.4	2.6	1.4	1.1	4.6	2.4	1.4
Xylose	1.0	0.5	1.3	0.3	1.5	0.9	0.5
Ribose	0.5	0.0	0.5	0.0	0.0	0.2	0.3
Fucose	0.0	0.0	0.0	0.0	0.0	0.0	0.0
(G+A)[d]	79.2	68.5	80.7	81.8	75.8	77.2	5.4
(G+A+Glc)[d]	90.3	92.5	92.8	93.1	91.3	92.0	1.2
S[d]	51.2	57.5	48.4	47.9	48.5	50.7	4.0
(G/A)[d]	1.60	1.64	1.77	1.76	2.13	1.78	0.21
(A/G)[d]	0.62	0.61	0.56	0.57	0.47	0.57	0.06
(S/G)[d]	1.05	1.35	0.94	0.92	0.94	1.04	0.18

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]N = needles, S = stems, R = roots. Indicates the parts of the seedlings from which the β-lectins were isolated.
[c]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[d]These quantities are derived from the data in the column under which they appear.
(G+A) = galactose plus arabinose.
(G+A+Glc) = galactose plus arabinose plus glucose.
S = the sum of the nongalactose sugars.
(G/A) = galactose divided by arabinose.
(A/G) = arabinose divided by galactose.
(S/G) = summed nongalactose sugars divided by galactose.

APPENDIX VI (Continued)

TABLE XXXII

PINACEAE β-LECTIN NEUTRAL SUGAR COMPOSITION
AS MOLE PERCENT OF THE TOTAL NEUTRAL SUGARS

Monosaccharide	Sample Number[a]			Species: Loblolly Pine Source: Callus (CL)	
	1	2	3	$\bar{X}$[b]	SD[b]
Galactose	53.2	56.3	46.2	51.9	5.2
Arabinose	30.8	29.0	25.4	28.4	2.7
Glucose	10.5	7.9	18.3	12.2	5.4
Rhamnose	4.4	4.3	4.0	4.2	0.2
Mannose	0.3	1.8	4.4	2.2	2.1
Xylose	0.8	0.7	1.7	1.1	0.5
Ribose	0.0	0.0	0.0	0.0	0.0
Fucose	0.0	0.0	0.0	0.0	0.0
(G+A)[c]	84.0	85.3	71.6	80.3	7.6
(G+A+Glc)[c]	94.5	93.2	89.9	92.5	2.4
S[c]	46.8	43.7	53.8	48.1	5.2
(G/A)[c]	1.73	1.94	1.82	1.83	0.10
(A/G)[c]	0.58	0.52	0.55	0.55	0.03
(S/G)[c]	0.88	0.78	1.16	0.94	0.20

[a]Each analysis represents a single determination on separate preparations of β-lectin.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation.
[c]These quantities are derived from the data in the column under which they appear.
(G+A) = galactose plus arabinose.
(G+A+Glc) = galactose plus arabinose plus glucose.
S = the sum of the nongalactose sugars.
(G/A) = galactose divided by arabinose.
(A/G) = arabinose divided by galactose.
(S/G) = summed nongalactose sugars divided by galactose.

APPENDIX VI (Continued)

TABLE XXXIII

PINACEAE β-LECTIN NEUTRAL SUGAR COMPOSITION AS MOLE PERCENT OF THE TOTAL NEUTRAL SUGARS[a]

Monosaccharide		Source Averages[a] DS	SS	CT	SP	CL	$\bar{X}$[b]	SD[b]
Galactose	D	63.4	50.5	46.9	41.2	47.6	48.9	9.2
	L	61.7	57.8	51.2	49.3	51.9	53.8	6.1
Arabinose	D	24.2	20.8	31.3	31.6	35.6	29.0	6.1
	L	24.9	26.8	32.1	27.9	28.4	28.0	3.2
Glucose	D	4.4	15.1	18.4	21.4	13.8	15.4	9.3
	L	6.3	6.7	10.3	14.8	12.2	10.6	5.2
Rhamnose	D	6.9	6.6	0.0	2.2	0.0	3.0	4.0
	L	6.0	6.5	2.4	4.5	4.2	4.7	2.3
Mannose	D	0.4	3.8	3.3	2.0	2.5	2.4	1.9
	L	0.6	1.5	2.8	2.4	2.2	1.9	1.4
Xylose	D	0.8	3.2	0.0	0.9	0.5	1.0	1.4
	L	0.5	0.7	0.5	0.9	1.1	0.7	0.4
Ribose	D	0.0	0.0	0.0	0.1	0.0	0.1	0.1
	L	0.0	0.0	0.3	0.2	0.0	0.1	0.3
Fucose	D	0.0	0.0	0.0	0.6	0.0	0.2	0.3
	L	0.0	0.0	0.4	0.0	0.0	0.1	0.2
(G+A)[a]	D	87.5	71.3	78.2	72.8	83.2	77.9	9.4
	L	86.6	84.6	83.3	77.2	80.3	81.8	5.7
(G+A+Glc)[a]	D	91.9	86.4	96.7	94.2	97.0	93.3	5.0
	L	92.9	91.3	93.6	92.0	92.5	92.4	2.2
S[a]	D	36.6	49.5	53.1	58.8	52.4	51.1	9.2
	L	38.3	42.2	48.8	50.7	48.1	46.2	6.1
(G/A)[a]	D	2.64	2.47	1.50	1.31	1.34	1.79	0.63
	L	2.54	2.15	1.60	1.78	1.83	1.95	0.42
(A/G)[a]	D	0.38	0.42	0.67	0.77	0.75	0.62	0.18
	L	0.41	0.47	0.63	0.57	0.55	0.53	0.09
(S/G)[a]	D	0.58	1.01	1.15	1.48	1.10	1.11	0.40
	L	0.63	0.73	0.97	1.04	0.94	0.88	0.21

[a]Tabulated from the X columns of the preceding ten tables, Tables XXIII-XXXII.
[b]$\bar{X}$ = Arithmetic average, SD = standard deviation. In this table, the average and its standard deviation are those of all the individual determinations made on each species, 17 for the Douglas-fir (D) and 17 for the loblolly pine (L).

APPENDIX VI (Continued)

TABLE XXXIV

DUNCAN'S MULTIPLE RANGE TESTS ON POOLED DATA

Monosaccharide	DS[a]	SS	CT	SP	CL	AOV(2)[b] Analysis of Variance		
						Sp	Dv	Sp x Dv
Galactose	62.5[c]	54.1[d]	49.0[d]	48.0[d]	49.7[d]	S	S	NS
Arabinose	24.5[d]	23.8[d]	31.7[c]	32.0[c]	32.0[c]	NS	S	S
Glucose	5.3	10.9	14.4	18.1	13.0	NS	NS[e]	NS
Rhamnose	6.4[c]	6.5[c]	1.2[d]	3.7[cd]	2.1[d]	NS	S	NS
Mannose	0.5	2.7	3.3	2.2	2.3	NS	NS	NS
Xylose	0.6[d]	1.9[c]	0.2[d]	0.8[d]	0.8[d]	NS	SS	SS
Ribose	0.0	0.0	0.1	0.1	0.0	ND[f]	ND	ND
Fucose	0.0	0.0	0.2	0.3	0.0	ND	ND	ND
(G+A)	87.0	77.9	80.7	75.0	81.8	NS	NS	NS
(G+A+Glc)	92.4[cd]	88.9[d]	95.1[c]	93.0[cd]	94.7[c]	NS	S	NS
S	36.6[d]	45.8[c]	50.9[c]	52.0[c]	50.2[c]	NS	S	NS
(G/A)	2.59[c]	2.31[c]	1.55[d]	1.51[d]	1.58[d]	NS	S	NS
(A/G)	0.39	0.43	0.64	1.51	1.58	NS	S	NS
(S/G)	0.61[d]	0.87[c]	1.06[c]	1.10[c]	1.02[c]	S	S	NS

[a]DS, SS, etc. as per Table II, Appendix II. Arithmetic mean of all the 17 values for a developmental state as mole percent of the total carbohydrate.

[b]AOV(2): two-way analysis of variance with interaction; S = significant and NS = not significant at the 5% level of probability. From left to right, the two main effects (species, Sp; development, Dv) and their interaction (Sp x Dv).

[c..d]Duncan's Multiple Range Test for a residue across developmental states; means followed by a common superscript are not different at the 5% level.

[e]Glc: AOV(2) on weight percent data gave development as a significant factor.

[f]ND = not determined.

n.b. = Symbols for neutral sugar parameters are as per Tables XXIII-XXXIII, Appendix VI.

APPENDIX VI (Continued)

TABLE XXXV

ARITHMETIC MEANS OF PINACEAE β-LECTINS

	Douglas-fir[a]		Loblolly Pine[a]			
	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}^b$	SD[b]
Galactose	140.7	64.1	189.3	40.3	51.3	8.1
Arabinose	79.9	32.4	99.3	27.7	28.5	4.8
Glucose	31.3	15.2	33.9	21.6	13.0	7.8
Rhamnose	11.0	16.9	17.0	9.5	3.9	3.3
Mannose	5.3	3.8	7.5	6.3	2.2	1.7
Xylose	2.5	2.6	2.5	2.0	0.9	1.0
Ribose	0.1	0.2	0.3	0.8	0.1	0.2
Fucose	0.5	1.1	0.4	1.2	0.1	0.3
(G+A)[c]	220.6	88.9	288.6	63.6	79.8	7.9
(G+A+Glc)[c]	251.9	89.0	322.5	77.4	92.8	3.9
S[c]	130.7	46.1	159.9	52.9	48.7	8.1
T[c]	271.4	99.1	349.2	86.0		
(G/A)[c]	1.79	0.63	1.96	0.42	1.87	0.53
(A/G)[c]	0.71	0.36	0.53	0.10	0.57	0.15
(S/G)[c]	1.09	0.43	0.88	0.21	1.00	0.34

[a]Data plotted in the histogram of Fig. 17. These data are on an absolute basis (μmoles of monosaccharide per 100 mg of β-lectin) and have been calculated from the 17 carbohydrate determinations on each species reported in Tables XXIII-XXXII.

[b]$\overline{X}$ = Arithmetic average, SD = standard deviation. These values are on the basis of mole percent of the total neutral sugars and have been calculated from the 34 individual determinations of Tables XXIII-XXXII.

[c]These quantities are the means of absolute-basis derived figures from the individual determination of Tables XXIII-XXXII.

(G+A) = galactose plus arabinose.
(G+A+Glc) = galactose plus arabinose plus glucose.
S = the sum of the nongalactose sugars.
T = the total amount of neutral sugar found.
(G/A) = galactose divided by arabinose.
(A/G) = arabinose divided by galactose.
(S/G) = summed nongalactose sugars divided by galactose.

APPENDIX VII

pH VALUES OF ELECTROFOCUSED COMPONENTS

TABLE XXXVI

ISOELECTRIC FOCUSING OF PINACEAE β-LECTINS

Band No.	LDS	DDS	LSS	DSS	LCT	LSP	DSP	LCL
1	3.0w	3.0	3.0	3.0w				3.0
2					3.4	3.4	3.4	
3		3.7w	3.7w					3.7
4					3.9	3.9	3.9	3.9w
5		4.0w	4.0w					
6		4.1w						4.1
7	4.25w	4.25w	4.25	4.25w				4.25
8	4.35	4.35	4.35	4.35	4.35	4.35	4.35	4.35
9			4.4w					4.40w
10	4.50w	4.50	4.50	4.50w				4.50
11	4.60	4.60	4.60	4.60	4.60	4.60	4.60	4.60
12		4.65w			4.65	4.65	4.65	
13		4.70w						4.70
14	4.80w				4.80	4.80	4.80	
15	4.85w	4.85	4.85	4.85w				4.85
16					4.95	4.95	4.95	
17	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
18	5.10w	5.10	5.10	5.10				5.10
19	5.15w		5.15w			5.15	5.15w	5.15
20	5.20w	5.20w	5.20w					
21					5.25	5.25	5.25	
22	5.30w	5.30w	5.30		5.30	5.30	5.30	5.30
23	5.35	5.35		5.35		5.35	5.35	5.35
24	5.40w	5.40w	5.40					5.40
25					5.45	5.45	5.45	

See end of this table for footnotes.

APPENDIX VII (Continued)

TABLE XXXVI (Continued)

ISOELECTRIC FOCUSING OF PINACEAE β-LECTINS

Band No.	LDS	DDS	LSS	DSS	LCT	LSP	DSP	LCL
26	5.50w		5.50		5.50	5.50	5.50	5.50
27	5.60w	5.60w	5.60		5.60	5.60	5.60	5.60
28	5.65w	5.65w		5.65w		5.65w	5.65w	5.65w
29					5.70	5.70	5.70	5.70w
30	5.75w	5.75	5.75w	5.75		5.75	5.75	5.75
31	5.80w	5.80w	5.80w			5.80	5.80	
32						5.85	5.85	
33	5.90w	5.90	5.90w		5.90	5.90	5.90	
34	5.95w		5.95w			5.95	5.95	
35	6.00w	6.00		6.00		6.00w	6.00w	
36						6.05w	6.05w	
37	6.10w		6.10					
38			6.15w		6.15	6.15w	6.15w	
39	6.20w		6.20w			6.20w	6.20w	
40	6.30w		6.30w		6.30	6.30w	6.30w	6.30
41	6.40w				6.40	6.40w	6.40w	
42	6.50w		6.50			6.50w	6.50w	6.50w
43	6.60		6.60			6.60w	6.60w	6.60
44	6.70w		6.70		6.70			6.70
45	6.80w		6.80			6.80w	6.80w	
46	6.90		6.90		6.90			6.90
47	7.00w		7.00w					7.00w

w = weak, represented by a dashed line in the previous figure.

APPENDIX VIII

SUMMARY OF β-LECTIN SPECIES DIFFERENCES

TABLE XXXVII

OVERVIEW OF PARAMETER VARIATIONS

Parameter[a]	n[b]	$\bar{X}$[b]	SD[b]	Min.[b]	Max.[b]	Range[b]	AOV(2)[b] Analysis of Variance		
							Sp	Dv	Sp x Dv
Sed. Coeff.	D(18)	6.39	0.81	4.93	7.94	3.01			
	L(22)	5.56	0.66	4.59	6.93	2.34			
	P(40)	5.94	0.83	4.59	7.94	3.35	S	S	NS
Protein, %	D(22)	11.3	9.8	1.6	30.2	28.6			
	L(26)	6.8	5.0	0.7	19.0	18.3			
	P(48)	8.8	7.8	0.7	30.2	29.5	S	S	S
Asx, %	D(22)	9.2	2.4	5.9	14.0	8.1			
	L(26)	11.9	4.2	7.0	22.5	15.5			
	P(48)	10.7	3.7	5.9	22.5	16.6	S	S	S
Hyp, %	D(22)	2.2	2.9	0.0	8.5	8.5			
	L(26)	0.8	1.1	0.0	3.4	3.4			
	P(48)	1.4	2.2	0.0	8.5	8.5	S	S	S
Gal, %	D(17)	48.9	9.2	32.3	67.4	35.1			
	L(17)	53.8	6.1	42.5	68.0	25.5			
	P(34)	51.3	8.1	32.3	68.0	35.7	S	S	NS
Nongal, %	D(17)	51.1	9.2	32.6	67.7	35.1			
	L(17)	46.2	6.1	32.0	57.5	25.5			
	P(34)	48.7	8.1	32.0	67.7	35.7	S	S	NS
S/G	D(17)	1.11	0.40	0.48	1.53	1.05			
	L(17)	0.88	0.21	0.47	1.35	0.88			
	P(34)	1.00	0.34	0.47	1.53	1.06	S	S	NS

See following page for footnotes.

APPENDIX VIII (Continued)

TABLE XXXVII (Continued)

OVERVIEW OF PARAMETER VARIATIONS

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[a]Parameters characterized:
Sed. Coeff. = sedimentation coefficient in Svedberg units.
Protein, % = protein as weight percent of the β-lectin sample.
Asx, % = aspartate content as mole percent of the total protein moiety.
Hyp, % = hydroxyproline content as mole percent of the total protein moiety.
Gal, % = galactose content as mole percent of the total neutral sugars.
Nongal, % = the sum of the nongalactose sugars (arabinose, glucose, rhamnose, mannose, xylose, fucose and ribose) as mole percent of the total reducible carbohydrate.
S/G = the ratio of nongalactose sugars to galactose (nongal, %/gal, %).
[b]Column headings:
n = the number of observations (single determinations on separate preparations of β-lectin) on the parameter for Douglas-fir (D), loblolly pine (L) and the pooled (P) species.
$\bar{X}$ = arithmetic mean, SD = standard deviation, Min. = minimum value for an observation.
Max. = maximum value for an observation, range = max.-min.
AOV(2) = results of the two-way analysis of variance with interaction.
S = significant, and NS = nonsignificant at the 5% level of probability. From left to right, the two main effects (species, Sp; development, Dv) and their interaction (species on development, Sp x Dv).